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ADNEXAL CARCINOMA OF THE SKIN *

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Much confusion exists concerning the classification and histogenesis of that group of tumors arising from the epidermis and its adnexa generally known as "basal cell epithelioma." Some of its members are noncancerous, while others are frankly, though not intensely, malignant. In his textbook on dermatological pathology, McCarthy [1] groups these under the heading "Basal Cell Epithelioma" and recognizes four types, all of which he considers benign. They are: (1) growths developing from groups of cells located beneath the epidermis or growing downward into the cutis as prolongations from it; (2) epithelioma adenoides cysticum of Brooke; (3) small, multiple, walnut-sized tumors appearing in groups and having a widespread distribution (morphologically intermediate between groups 1 and 2); and (4) cylindroma. Discussing group 3, he indicates that its members arise in a multicentric fashion and exhibit a definitely cystic architecture which is fundamentally that of "basal cell epithelioma"; that is to say, it presents masses of pleomorphic cells packed into spheroidal cores that are enclosed by a membrane of radially arranged basal cells. Many of these masses undergo cystic degeneration.

Such a classification is purely morphological and the student or young pathologist reading it will find himself much confused when he turns to other books or articles and discovers the "cylindroma" referred to as "Brooke's tumor," or as "hydradenoma solidum." The tumors of McCarthy's groups 1 and 3 are called "basal cell carcinoma" or "hair matrix carcinoma" according to the views of the particular authority consulted. Thus it is obvious that the student's confusion is quite natural and one must infer that our knowledge of these growths is equally confused, as well as faulty. It is the purpose of this paper to attempt to bring some order out of the existing muddle.

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Historical Review

At the risk of being prosaic it will be necessary to trace the history of the theories as to "basal cell tumor." It was first christened "the gland-like carcinoma of the superficial epithelium, carcinoma epitheliale adenoides," by Krompecher [2] in 1900; in 1903 he published a monograph entitled "Der Basalzellenkrebs," and expanded his conception of an origin in basal cells of the epidermis to include as well those of the epidermoid mucosa of the oral cavity, nasal mucosa, larynx, and similar regions.[3] He recognized four types of growth; solid, pouch-like downgrowths from the epidermis; epithelial masses containing cysts; glandular forms composed of interlacing bands of epithelium; and nests of epithelium that produced parakeratotic pearls. He considered origin in hair follicles, sebaceous glands and sudoriparous glands, which is interesting in view of what is to follow in this paper. Unfortunately, in the later monographs, he condensed these possibilities into what might be considered an oversimplified probability: origin of all these tumors, including those of epidermoid and schneiderian mucous membranes, from the basal layer of germinal cells.

There the matter has rested for almost 45 years, and the vast majority of pathologists and dermatologists speak of basal cell carcinoma as an undisputed entity. Holding to this view, they are forced to explain certain incongruities in the behavior of the tumor. Why should it be only mildly malignant if it be derived from the least differentiated and the most primitive cell of the epidermis? The apparently better differentiated epidermoid or squamous cell carcinoma is more malignant; why is that the case? Again, why is the basal cell carcinoma a trivial affair in the skin, while it is notoriously malignant when it arises from the epidermoid mucosa of the oral cavity or esophagus? Lastly, why should this basal cell tumor take on a regularly characteristic architecture that bears no close resemblance to epidermis and often consists simply of two types of cell? This latter form is pathognomonic of the so-called "basal cell carcinoma." One is impelled to ask, in the vernacular, "how do basal cells manage to get that way?"

In 1910 Mallory [4] demonstrated fine longitudinal fibrils in the cells of the tumor which were identical in their appearance with similar fibrils in cells of embryonal hair follicles and quite unlike the intracellular fibrils of the prickle cells of the epidermis, the normal first-born offspring of the basal cell. From this he deduced that the tumors arose from the cells of hair matrices, sebaceous and sudoriparous glands. He believed that the basal cells of the epidermis were early forms of prickle cells and had the same type of intracellular fibrils, filaments that run in all directions and are intercellular as well as intracellular. Unfor-

tunately, his findings were limited to these fibrils and his report constituted a small part of a lecture on "Recent Progress in the Microscopic Anatomy and Differentiation of Cancer," so that his theory on "hair matrix carcinoma" was buried in an article, the title of which gave no clue to its presence. In 1914 he published his "Principles of Pathologic Histology," and in it he restated the theory and mentioned the tumor as a "hair matrix carcinoma" which had its origin in the hair matrices. He did not document this opinion in any way other than to call attention to the fibrils mentioned above.[5] There were, therefore, two reasons for the reluctance of the public to accept his theory: insufficient published documentation, and the idea that the tumor arose in the hair matrix, which is situated in the bulb and with which the tumors show no direct connection. In his conversation with his pupils, however, he made it clear that he believed that the tumors were of adnexal origin.

In an article written practically at the same time that McCarthy [1] published his textbook (1931), Haythorn [6] reviewed the subject in The American Journal of Cancer, confining his observations entirely to the "basal cell carcinomas." He said: "If we are correct, the neoplasm now regularly called 'basal-cell carcinoma' has less real claim to that name than any of the tumors to which the term was originally applied." He referred, of course, to Krompecher's monograph.[2] As a pupil of Mallory, he approached the subject from the hair matrix angle and adhered to Mallory's opinion that the tumor is derived from hair matrices. Haythorn made a very careful examination of a large number of these carcinomas and his article is excellently documented by photomicrographs illustrating all their varieties. He first considered the matter of apparent downgrowth of plugs of epithelium from the basal epidermal layer and found that continuity between the cells of these plugs and those of the basal layer was only apparent; there was usually a demonstrable, sudden change in the type of the cells where prickle cells were replaced by elements containing the diagnostic longitudinal fibrils of Mallory. He stressed the point that epidermal basal cells are immature prickle cells and demonstrated with photomicrographs more or less rudimentary hair shafts in the cellular nests of the carcinoma. He cited Broders' statement [7] that he had found but one example of this in a series of 252 tumors which he had examined; this he considered to be an understatement.

Haythorn placed much emphasis upon the structure of the membrana propria of the tumor's complexes and investigated it in sections impregnated with silver. He found that it resembled the connective tissue elements of the hair follicle rather than epidermal basement membrane.

Exploring the glandular varieties of the growth, he came to the conclusion that they might be derived from sebaceous glands, which are (after all) merely adnexa of the pilar follicles. He found insufficient evidence of connection with sudoriparous glands to warrant entertaining the assumption that they might be involved in the production of these carcinomas. Nevertheless, on page 1995 of his article,[6] he published two photomicrographs which he said represented a very rare picture; they strongly suggest sudoriparous glandular structure.

In his book on diseases of the breast, Geschickter [8] makes the statement: "The basal-cell cancers of the skin which are adenocystic in type are subepidermal lesions arising from appendages of the skin such as the sudoriferous or salivary glands or the pilosebaceous apparatus." The statement is quite categorical and coincides with the thesis of this paper, excepting that he includes the salivary glands as possible sites of origin of "basal cell carcinoma."

In 1943 Gates, Warren, and Warvi [9] discussed the neoplasms originating in sudoriparous glands; their article is excellent and covers the subject of the nonmalignant varieties very well. It will be noted that they now classify the former "cylindroma" as a solid hydradenoma. This has a striking similarity in its histological architecture to that of a quiescent and very orderly "basal cell carcinoma." It is noteworthy that the two may be closely associated, and hence possibly equally intimately related one to the other. Haythorn [6] cited Owen [10] as having presented the theories of histogenesis of basal cell carcinoma under the name of the authors who have advanced them—

From basal cells: Ewing [11] (and with him the majority of authorities*);

From hair matrices and sebaceous glands: Mallory,[5] Hernaman-Johnson,[12] Paul,[13] Walker [14] (and Haythorn [6]);

From seborrheic patches or senile keratoses: Blasdel.[15]

Investigation

For many years I have been curious as to which of these theories is the correct one and have been planning to work out some scheme that might serve to elucidate the peculiarities of the "basal cell carcinoma," which will be called "adnexal carcinoma" in the ensuing pages of this text. In the absence of experimental proof, one is forced to fall back upon the interpretation of transitional pictures and the frequently faulty deductions derived therefrom. An experimental approach to the subject is difficult and, at the present writing, is impracticable in our laboratory. The possibility of injecting carcinogenic agents intra-

* Parenthetical additions are by the author.

dermally and intrafollicularly in appropriate animals is self-evident, but carrying out this procedure should include injections into sudoriferous ducts, and most laboratory animals have none. It has, therefore, been decided to attack the problem in the old-fashioned way and to depend upon histological study of representative sections and an interpretation of the findings thus accumulated.

GENERAL REVIEW OF MATERIAL

All examples of adnexal carcinoma available in the collection of the department since 1932 were subjected to a preliminary microscopical survey. There were well over 200 of them and they included every type. After screening out and rejecting the larger tumors as being unpromising of anything new, the sections from the smaller and early growths were selected for intensive examination. The study confirmed the findings of Mallory [4,5] and of Haythorn [6] in almost every particular.

Starting from the assumption that many of the tumors might be of sudoriparous origin, on account of the striking resemblance between adnexal carcinoma and solid hydradenoma and because of the development of the former in an example of the latter recently received, it was immediately evident that far more of them exhibited a definite, if distorted, analogy to pilar units. There was a great variation in the degree of differentiation, some presenting striking similarity to pilar units while others were much further removed from the normal pattern of these, and one was forced to search long for any resemblance. As there appeared to be a group that more closely simulated sudoriparous glands and hydradenomas, these were segregated from those which showed an analogy to pilar or sebaceous units. Pigmented varieties were also sequestered and a few that gave evidence of truly basocellular architecture were likewise set apart.

Description of Types

Several moderately distinctive types were recognizable after a survey of the series and these were arranged, as they are described below, in groups that might indicate their origin rather than afford only morphological differentiation, which means little.

I. Pilar Type

Roughly three-fourths of the sections represented the pilar type. When this tumor is studied microscopically it is noted that the overlying epidermis is more often atrophic than hypertrophic; the hairs in the region are either deformed or absent, many are crowded to one side and compressed in the adjoining area. Oftentimes they are stretched

out into long, thin structures that skirt the margin of the growth in a parallel arc. The absence of hairs in the skin overlying an adnexal carcinoma might be interpreted as a result of the absorption of hairs into the substance of the growth. (This seems to be true of the Wagner-Meissner corpuscles in the papillae overlying a melanoma; they appear to become incorporated in the neoplasm and to disappear from the derma in its neighborhood.) The pilar type represents several subvarieties depending upon their degree of differentiation, or the amount of degenerative changes that present. They are probably prognostically as well as academically important and are not based solely upon structure.

1. *Pilar Type Proper.* The pilar type proper imitates the architecture of hair follicles in an unmistakable manner (Fig. 1): There is visible evidence of differentiation into forms that correspond closely with those of normal hair follicles. As one studies the composition of such formations, it is seen that there is a basal layer (possibly manifesting vesiculation) within which there are concentrically arranged islands that imitate various elements of the normal hair follicle. At the core there may be clumps or masses of keratinized material that is much like that of the hair shaft. That Broders found them so seldom is strange; in our sections, stained by the Masson-Goldner method,[16] they took on a bright orange hue and were found in a large number of the tumors examined. These neoplastic complexes may exhibit much of the conventional outline of pilar units, or they may present many sinuous, bulbar outgrowths or processes suggesting pseudopodia. Observation of this well differentiated type of tumor leaves one convinced of its relationship to hairs.

2. *Primordial Type.* In the primordial type the cells form large spheroidal, conical, or ovoid masses comprising two cellular units: a peripheral delimiting single layer of radially arranged basal cells and a central core of polymorphic elements which are scattered in a haphazard fashion. Such a complex might be compared with a "filled" stone wall; the outer layer of cells is the "face" of matched and fitted masonry, while the core constitutes the "fill" of piled-in, rough stones. The primordial type is very common(Fig. 2) in the earlier forms of the adnexal carcinoma and it may exhibit multicentric origin from the basal layer of the epidermis. It is the prototype of the miscalled "basal cell tumor."

3. *Cylindrical-Celled or "Ribbon" Type.* The cylindrical-celled or "ribbon" type is rare and consists of rosettes and complex festoons of ribbon-like bands comprising a layer of cylindrical and enlarged cells quite unmixed with any other varieties. Here the "wall" is entirely composed of matched and fitted masonry (Fig. 3).

4. Cystic Type. Rather than a true type, the cystic group constitutes a variant of types 1 and 2 in which there has been vacuolization of the cells with a fusion of the vacuoles that produces cystic cavities; or in which there has been lysis of the stroma with the production of cysts. Both processes may be seen in the stages of development. It is possible that the former, in which the cells become vesicular, represents a change of pilar to sebaceous elements.

II. Sudoriparous Glandular Type

When the sudoriparous glandular type of adnexal carcinoma is present, it is noteworthy that there are few sweat glands in its neighborhood and that those which persist are crowded downward into the derma and subcutaneous tissue. One cannot find bits of hair in these tumors. Instead, there are small onion-like bodies (misinterpreted as epithelial pearls) which are composed of concentrically arranged squamous cells, sometimes containing droplets of keratohyalin and surrounding a tiny lumen that occasionally is lined with a thin layer of eleidin. Such a lumen may expand into a small cyst loosely filled with keratinized scales, and it is then difficult to distinguish from an abortive hair. However, such cysts are frequently noted developing in the terminal, intra-epidermal portion of sudoriferous ducts. The bulk of these growths is composed of complexes of cells arranged in double rows, like imperfect ducts or tubules (Fig. 4). Myo-epithelium may be demonstrated at the margins of the complexes in many instances. Two subtypes are recognized.

1. Adenoid Type. The adenoid type has just been described.

2. Hydradenomatous Type. The hydradenomatous type appears in two forms: (a) A form resembling the solid hydradenoma ("cylindroma"), but manifesting less differentiation and many mitotic figures. Possibly it represents the malignant form of solid hydradenoma (Fig. 5). (b) A type resembling the papillary cystic hydradenoma and, less definitely, the apocrine sudoriparous gland during menstrual stimulation. This is not often seen and it may readily be mistaken for a seborrheic verruca.

III. Basal-Celled Type

As already stated, the basal-celled type is uncommon and, unless it be observed in connection with one or another of the forms just listed, it would be more properly considered to be a plexiform epidermoid carcinoma (Fig. 6). It may occasionally be one, since epidermoid carcinoma frequently develops in the margin of rodent ulcers, the advanced stage of adnexal carcinoma. It is composed of elongated, fusiform cells that grow downward from rete pegs and disrupt them in the process,

later to anastomose with one another in the derma. Here they constitute a plexus of undifferentiated cells which may sometimes exhibit knots, or thickenings of better differentiated elements, and thus acquire more similarity to the pilar type of adnexal carcinoma.

Innervation of the Tumors

Several years ago, while experimenting with the impregnation of neurofibrils with silver, I noticed that the Nonidez modification of the Ramon y Cajal method [17] demonstrated plexuses of very prominent and readily visible nervous filaments in the pilar papillae and about the sebaceous glands. These are described in textbooks of histology. It has been pointed out that they are apparently concentrated in these situations. It is also evident that there are abundant, but finer neurofibrils coursing in the stroma and about the tubules of the eccrine sweat glands. Very few, if any, such fibrils are demonstrable in the papillae of the derma, or in the basal layers of the epidermis.

These facts led to the impregnation of a few small adnexal carcinomas by the above method. The results were so striking that they may constitute the subject matter of a future article. It was found that there are heavy bundles of nonmyelinated fibrils running in the stroma of adnexal carcinomas (Fig. 7). They may arise from the larger trunks of myelinated fibers that abut on the growth. They send a few filaments into the masses of neoplastic cells, where they soon become untraceable; some of them appear to end in terminal buds, or club-shaped extremities similar to nerve endings. While the usual plexuses are found about the normal follicles and sebaceous glands, as well as the sudoriparous glands in the sections, careful examination of the epidermis fails to reveal similar structures.

In the presence of this rich neural plexus in the neoplastic stroma we have further indication of its relationship to hairs, sebaceous glands, and sweat glands, rather than to basal elements of the epidermis in general. The tumor apparently acquires this neural apparatus much the same way as do the adnexal elements of the skin during their embryological development, but this is admittedly pure conjecture.

Pigmentation

Any of the enumerated types of adnexal carcinoma may exhibit more or less pigmentation which may be so intense as to produce a tumor that is grossly identical in its appearance with a malignant melanoma, being quite black and indefinitely outlined. One such tumor developed in a solid hydradenoma of the forehead that had been present for several years in an apparently quiescent condition. The matter of pig-

mentation has been discussed at length by Haythorn.[6] Nine of the 200 or more specimens collected in our department showed more than moderate pigmentation. The melanin is produced by the cells of the tumor and much of it becomes transferred to the cytoplasm of melanophores in the stroma. In dermal sections impregnated with silver, silver-positive melanin is most abundant in the basal layer of the skin, but it is found to extend a considerable distance up the necks of the hair follicles as well.

Arrectores Pilorum

Each hair has its small, smooth-muscular erector; arrectores pilorum may be found scattered throughout adnexal carcinomas. They may have been engulfed by the tumor, but their arrangement hints that they have some relation to the distorted hair follicles that constitute the pilar form of the neoplasm. This is not very strong evidence in support of my theory, but it is at least interesting to note that the small muscles are not much displaced nor destroyed by the tumor.

Investigation of Early Forms of the Tumor in Serial Sections

As it seemed unlikely that anything new might be added to the present conceptions of the histogenesis of this tumor through a morphological study of full-blown examples, two small and very early examples were chosen for investigation. The first was about 5 by 3 mm. in size and came from the nose of a woman in her fifth decade; the second was a very tiny lesion on the aural pinna of an elderly man. A preliminary study of sections indicated the advisability of cutting a series of them and then reconstructing selected areas in the tumor in three dimensions by means of models.

Method of Reconstruction. Accordingly, both tumors were laid down in serial sections and these were studied for suitable foci for tridimensional reconstruction. This was carried out satisfactorily and expediently by projecting the sections seriatim upon ordinary cardboard filing cards and outlining in pencil the structures to be built up, numbering the outlines consecutively. A complex was selected in each tumor and projected in the desired magnification. After making the outlines, the complex was measured on the microscopical section with an ocular micrometer and the figures were divided into those taken from the reconstructed model in centimeters. This gave the magnification. As the sections were cut at 6 μ, it was merely necessary to multiply the magnification by 6 in order to find out the requisite thickness of the lamina to be built up from the filing cards, measured in microns. It was found that one card 0.25 mm. in thickness was suitable for a magnification of about $\times$ 40. No attempt was made to adjust the

thickness of the cards with extreme accuracy. If the magnification was × 70 or × 80, two cards were used, the original cut-out being pasted upon another card which was then trimmed to the size and shape of the cut-out. The reproductions were intended to indicate the tridimensional structure in a general way, not to reproduce it exactly. The reasons for this will be clear when the reader has referred to the photographs of the models. The method is quite simple and it obviates the necessity for working with carefully prepared wax sheets. Furthermore, the preparation will not melt if placed in a warm spot. When the outlines were finished, they were cut out with scissors and pasted together in the proper sequence. After the glue had dried they were modelled by painting on molten paraffin and beeswax with a camel's-hair brush until a smooth layer of the mixture had covered the model completely, smoothing out its inevitable inequalities. The model was then scraped with a scalpel after the paraffin coating had congealed and painted in two colors of quick-drying enamel over shellac, the tumor in vermilion and the rest of the model in light blue.

Study of Models. Study was not limited to the finished models, for as each section was glued into place the diagrammatic outlines on its surface were observed and the manner of growth of the tumor became much more obvious than any bidimensional sections could render it. Comparison of doubtful areas in the model with the microscopical sections from which they were drawn enabled the correction of any misconception. Several points became manifest. The growth appears to begin as a loosening of elements of the rete in the neighborhood of the debouchment of the hair sheaths and sweat ducts through the epidermis. Thus cells accumulate until small nodules are produced, but these lie just *outside* or above the basal layer of cells and comprise a welter of polymorphic elements that exhibit numerous mitotic figures. These complexes appear to push the basal layer downward, bowing it out into the dermal connective-tissue (Fig. 8). In this way, conical or bulbous mammillary processes are formed in the derma without producing any corresponding distortion on the surface of the epidermis. Larger examples of these processes tend to mushroom out below the epidermis and gradually to take on bizarre forms not unlike those of certain tubers. They are usually covered by smaller nodose or bulbar processes that stud their surfaces and remind one of the sebaceous buds along the shaft of a pilar primordium, or even of small hair follicles. The large tuberous complexes of the tumor are attached to the epidermis by a relatively small and short stalk which represents the original conical or bulbous beginning of the growth. Such a stalk supports the complex shown in reconstruction 1 (Figs. 9 and 10) and is shown in microscopi-

cal sagittal section in Figure 18. The greatest over-all longitudinal diameter of this growth was about 2,465 μ; its width, 820 μ; and its thickness as reproduced, 570 μ. There was more of the tumor in sections that were cut and discarded before the serial sections were prepared, so that it was really somewhat thicker than this. The flattened pyramidal pedicle that supports the complex has a diameter of 799 μ at its widest point, but it is only 210 μ in thickness. The pedicle arises from the epithelium in the immediate vicinity of the neck of the pilar unit shown in the model. It will be noted that the section portrayed in Figure 18 shows the complex to be of the primordial type that exhibits considerable differentiation toward the pilar variety. Variations in the size and shape of the neoplastic complexes are observed; sometimes they are merely small conical projections which have not as yet grown into the larger variety; sometimes there are small areas of anastomotic columns of basal cells, but these are few in the early forms of the growth.

Interpretation of Reconstructed Tumor. Interpretation of these phenomena is admittedly hazardous. The cells that form the initial masses between the basal layer and the rete might be produced by the multiplication and emigration of basal elements, or by the multiplication of the pleomorphic central cells *in situ*. The latter possibility seems to be the case, as can easily be determined by noting the much larger number of mitotic figures among those cells as compared with the elements of the basal layer. They abut on prickle cells on the one hand and basal cells on the other, merging imperceptibly with either type along the border of the mass. A few prickle cells may lie among them; as for the basal cells, they appear to be forced downward by the accumulation of pleomorphic cells until they are pushed into the depths of the derma. They usually maintain their continuity with the basal layer of the epidermis, constituting the envelope of cells already described. This accounts for their radial orientation in respect to the tumor complex. Examined under high magnification, the cells of the central mass have little to distinguish them from any other primitive pleomorphic cell until they undergo differentiation. When this occurs, they mimic elements of the hair follicle, sebaceous, or sweat gland and do not produce prickle or squamous cells that typify the epidermis.

The cellular structure of the large, tuberous complexes is too familiar to delay us here; it has been described by many writers since Krompecher's time. Their observations as to occasional differentiation of a varying degree, the production of abortive or misshapen hair shafts, and the formation of cysts are all readily confirmed. One point has been overlooked, or underemphasized: the tendency of some of the

neoplastic complexes to send forth occasional tubular processes reminiscent in their shape of the ovipositors of certain fish or insects. Examination of these abortive tubules, which project only 1 mm. or so into the derma and then end abruptly in a tapering tip, shows that they bear an extraordinary histological likeness to sudoriferous ducts. Such a structure is shown in reconstruction 2 (Figs. 11 and 12), much enlarged and dominating the model. A microscopical section through it is illustrated in Figure 13. Another smaller abortive tubule is partly hidden behind the large hair follicle in reconstruction 1. Sometimes an even more abortive beginning of such ducts may be noted: a group of cells sprouts an elongated mammillary process from the surface of one of the larger tumor-complexes, forms a short prong that appears in three or four serial sections, and is then lost. On reconstruction it amounts to little more than a tiny and somewhat fusiform spike, or elongated "tear drop."

Discussion

Hypotheses

An attempt to explain the histogenesis of adnexal carcinoma on the basis of the data thus accumulated will, of necessity, be somewhat didactic. Let us consider what might be expected of the neoplastic elements were they primarily epidermal basal cells (as has for so long been claimed) and were they subjected to carcinogenic stimuli: what would a basal cell do under such circumstances? Normally it differentiates into prickle cells, next granular elements, and finally into keratinized squamous cells. Should it pursue this line in a tumor, it would pile up keratinized scales on the surface and produce a verruca; should differentiation stop in the prickle cell phase, the result would be acanthosis and a condyloma; should it fail to differentiate at all and remain of the basal cell type, it would divide and multiply to form a totally undifferentiated, plexiform tumor, the plexiform epidermoid carcinoma. It would not produce dermal adnexa, however, unless it was a certain specialized basal cell that was destined to do so. This will be discussed later.

Could basal cells form anything like this tumor, the adnexal carcinoma, and if so, where and how? In the embryo there are certain areas where basal cells become specialized to produce epidermal adnexa: hair, sebaceous and sudoriparous glands. During the process of doing so they form primordia that are strikingly similar to the structures that typify adnexal carcinoma (Figs. 14, 15, and 16).

Histogenesis and Histology of the Hair

The embryonal primordium of the hair is merely a downgrowth of two types of cell from the epidermis: an outer layer of radially orientated cylindrical elements and a core of polymorphic cells of an indifferent type, the *embryonal* hair matrix. From the sides of the plug thus formed small lateral buds are given off; they may develop into sebaceous glands, or they may be resorbed by rearrangement of their elements and thus disappear (Figs. 14 and 15). The hair that results from the differentiation of this simple primordium is, in effect, simply a column of invaginated epidermis. The keratinized layer is molded into a hair shaft, the granular layer persists for some distance up the sheath, the malpighian layer becomes altered to form the cells of Huxley and Henle, while the basal layer undergoes vesiculation until most of its cells are converted into cylindrical sacs. In the base of the bulb is a small group of undifferentiated cells which renew the shaft and constitute the *adult* hair matrix. Around the cellular sheath is a prominent reticular membrane with its cells lying parallel with the contour of the bulb and shaft; this begins near the opening of the sheath, surrounds it and the bulb, but is not comparable with the epidermal basement membrane which is more delicate, more reticular, and less compact. At the tip of the bulb there is an indentation into which a papilla of connective tissue and neurofibrils is received. No attempt is made here to detail the structure of the hair further, but it should be pointed out that among the 200-odd tumors examined, some analogy to the above outline was found in the majority of them. Abortive hair shafts, small collections of cells with keratohyaline granules, vesiculation of the basal cells surrounding the tumorous masses, and rudimentary papillae of connective tissue and nerves projecting into indentations in these masses; all of these were noted. The fact that the more primitive primordium was frequently imitated has been sufficiently indicated.

It seems that this similarity is not fortuitous, but that the tumor is merely recapitulating, in its neoplastic and disorderly way, the embryological process that precedes the formation of the dermal adnexa; in some instances it gets no farther than the embryonal phase, while in others it nearly succeeds in producing hairs. That the tumor arises in the *embryonal* rather than in the *adult* hair matrix is a more satisfactory explanation than Mallory's, as the former is situated exactly where these tumors appear to originate, rather than near the hair matrix of the bulb with which I could observe no direct connections. It is unfortunate that there is no definite explanation as to why embryonal primordia should persist in adult epidermis. Lacking direct evidence,

we must fall back upon one of two hypotheses: Virchow's ancient theory of cellular rests, or a retrogressive change in certain basal cells near the hair follicles, whereby they are returned to an embryonal or proliferative state.

Histogenesis and Histology of Sebaceous Glands

While examining the more glandular types of adnexal carcinoma one will occasionally be found which exhibits vacuolization of its cells to a degree that indicates sebaceous origin. Bearing in mind the fact that the tumors often arise at a point where the sebaceous ducts empty onto the surface, it would not be strange if these were included in the mechanism of the histogenesis of adnexal carcinoma. Sebaceous glands may be dismissed with the remark that they develop from the pilar primordia, as already indicated. Their ducts are connected with lobules of large cells containing lipid material that is discharged by the holocrine disintegration of the cells. These glands are usually very numerous in the neighborhood of adnexal tumors, possibly because of the fact that the latter most usually develop on the nose or upon parts of the face where there is much sebaceous secretion.

Histogenesis and Histology of Eccrine Sudoriparous Glands

It will be unnecessary to go into the finer differences between eccrine and apocrine glands, the former being much more pertinent to the subject under discussion. An eccrine sudoriparous gland is a very simple affair that develops by a downward growth of a conical primordium from the base of the epidermis. This type of primordium is common to the hairs, sweat glands, and the mammary glands. In the case of the sudoriparous variety, it tends to mushroom out under the epidermis as it grows, and from its apex a slender stalk of cells works its way downward into the corium or subcutaneous tissue and coils itself into a globose knot. The larger mass of primordial cells persists for a time and then disappears (Fig. 16). The epithelium of the sweat glands is simple, cuboidal and unspectacular. In the proximal, secreting portion of the tube there are two layers of cells, an inner cuboidal layer and an outer stratum of strap-like elements, the "myo-epithelium." The secretory tube becomes more and more compact as it approaches the epidermis and its cells begin to show granules of keratohyalin. Finally, the duct is a simple spiral channel in the keratinized layer of the epidermis, its lumen lined with a layer of eleidin. In the region of the coil, neurofibrillae are numerous and surround the tubules.

In those tumors which might be attributed to such glands it is possible to find small concentrically whorled structures with a tiny lumen

also lined with eleidin; it is well to note that a cross section of a sudoriferous duct as it joins the epidermis is almost identical in its appearance with these small structures. The production in the tumor of teat-like processes resembling sudoriferous ducts has also a conspicuous bearing on this argument, as well as the presence in many of the growths of a peripheral layer of myo-epithelium about the neoplastic complexes. To these points should be added the presence of a rich nervous supply in the stroma of the tumor and of sweat glands alike.

Metastases

It is well known that these tumors seldom metastasize and only then when they are large and extremely ulcerated; only one in our collection has done so. This was a large subaural rodent ulcer. A study of the metastases in lymph nodes is of value as it represents the trend of development in the tumor when this has been completely removed from its natural environment. It is found that the metastasis adheres closely to the basic pattern of the primary growth; it forms the same type of complexes and, in the instance cited, took on the pilar type of the parent tumor (Fig. 17). Were such a growth composed of undifferentiated basal cells of the epidermis, the metastasis should present nothing more than a plexiform arrangement of fusiform epithelial cells.

Recapitulation

As may be seen from the preceding pages, correlation of the various types of adnexal carcinoma and certain phases of the life history of the dermal adnexa is not a difficult matter. The primordial type of this tumor reproduces distorted pictures of the embryological development of the hairs, sebaceous and sudoriparous glands. Whether every adnexal carcinoma begins as a focal multiplication of primordial cells in the region of the mouths of the hair follicles, or whether some of them may grow directly from distorted adnexal elements is hard to determine. It is also probable that the adnexal carcinoma may develop from more than one type of primordium, as one tumor may show areas that indicate pilar origin and other areas that point towards a sudoriparous genesis. It is true, however, that in all of the very early examples of the tumor examined in this series, the formation of distorted primordia was the outstanding feature.

That these tumors do not arise from basal cells as such is indicated by the fact that they fail to differentiate along epidermoid lines. Their differentiation is entirely in the direction of pilar, sebaceous, or sudoriparous structure. It is the chief purpose of this article to divert attention from the epidermal basal cells toward those specialized basal cells

which form adnexal primordia. Secondarily, it is desirable to change the emphasis on the hair matrix to one on the adnexal primordium. In a general sense these tumors are derived from basal cells, as are all epidermal tumors, but this is no reason for calling them "basal cell carcinoma"; an epidermoid carcinoma would have just as much claim to that name.

The currently widely maintained concept that the adnexal carcinomas are composed of undifferentiated basal cells is quite incomprehensible to me; even a cursory examination of sections from one of them will prove that there is much differentiation going on in most of the neoplastic areas. In some tumors it is merely toward the embryonal primordium, which is the least specialized in its architecture; in others the trend is toward something reminiscent of dermal adnexa; while in a small group the differentiation falls just short of the production of such adnexa.

Conclusions

A study of the structure of early examples of "basal cell carcinoma" indicates that this neoplasm originates in distorted primordia of dermal adnexa rather than from ordinary epidermal basal cells. The tumor imitates the embryonal development of hairs, sebaceous and sudoriparous glands. The reasons for these assumptions are as follows:

1. A recapitulation of the histogenesis of dermal adnexa is present in the majority of the tumors.

2. There is a distinct similarity in the architecture of the growths to that of these adnexa in their adult state:

   (a) Abortive hairs are formed.

   (b) Abortive sudoriferous ducts may be observed in some instances.

   (c) There is a copious and complex development of neurofibrillae in the stroma of the tumors, comparable only to that seen in adult, normal adnexa.

3. The indications are that a given adnexal tumor may take origin from any or all three types of adnexal primordia.

4. Metastases in lymph nodes undergo the same type of differentiation and form the same abortive adnexal structures as are found in and produced by the primary tumor from which they have come.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

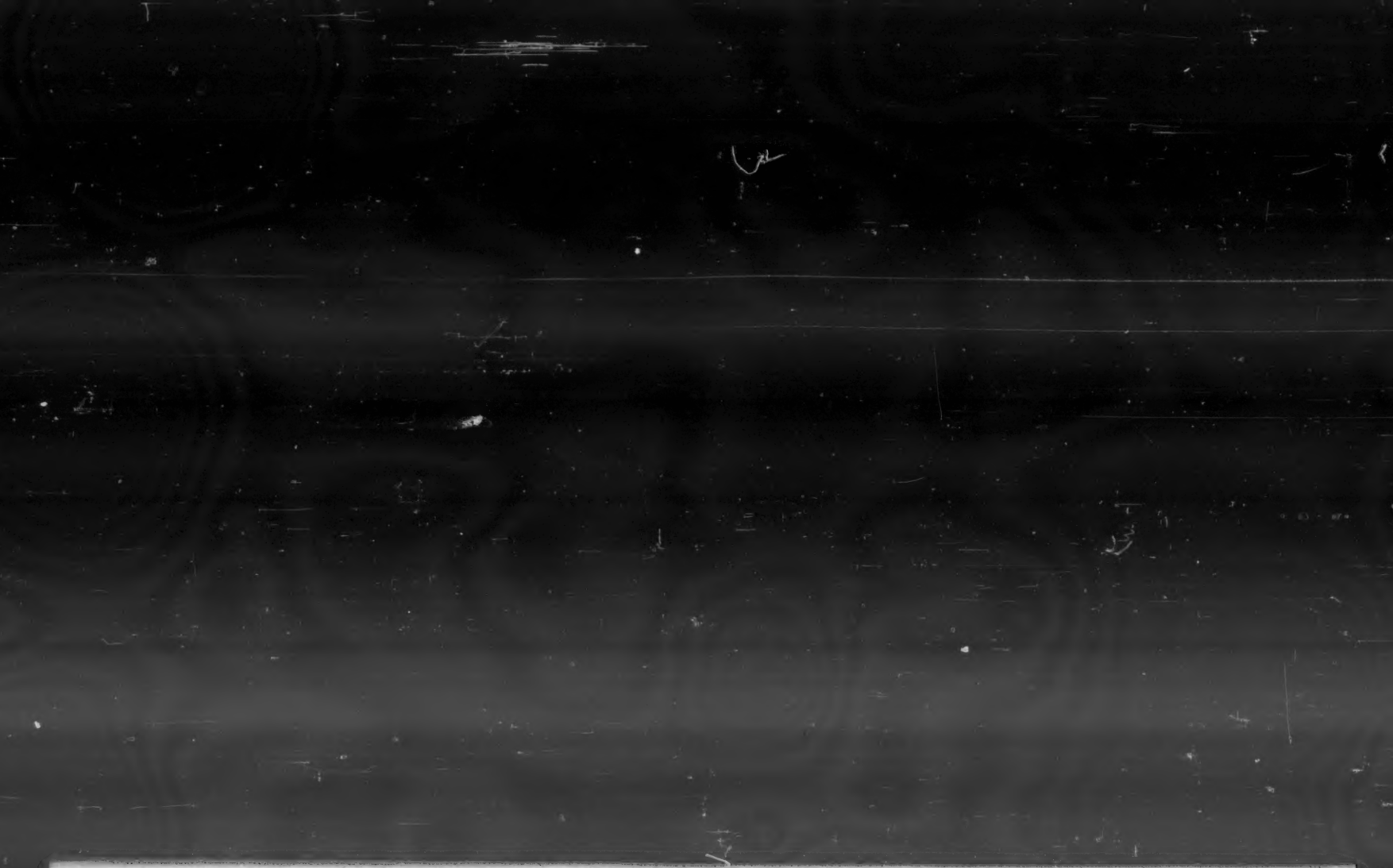
PLATE 1

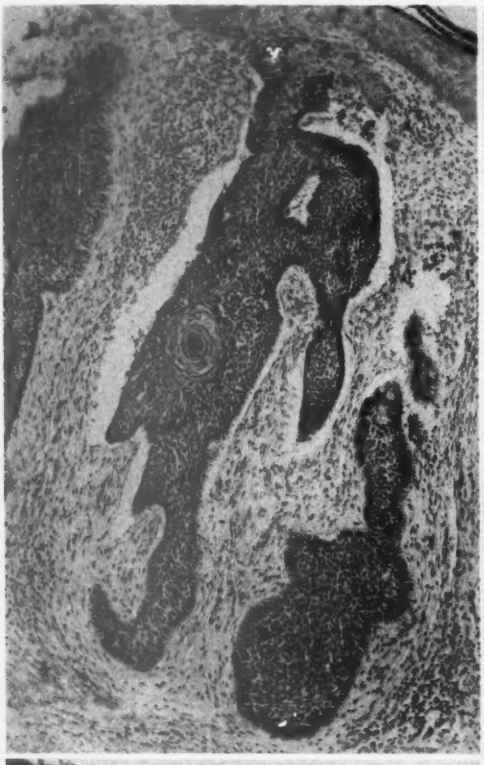
FIG. 1. True pilar type of adnexal carcinoma. Of note is the general resemblance of the central complex of cells to an embryonal pilar primordium. For comparison with Figure 15. Concentrically arranged cells at the center of the field simulate those of the neck of a hair sheath. × 90.

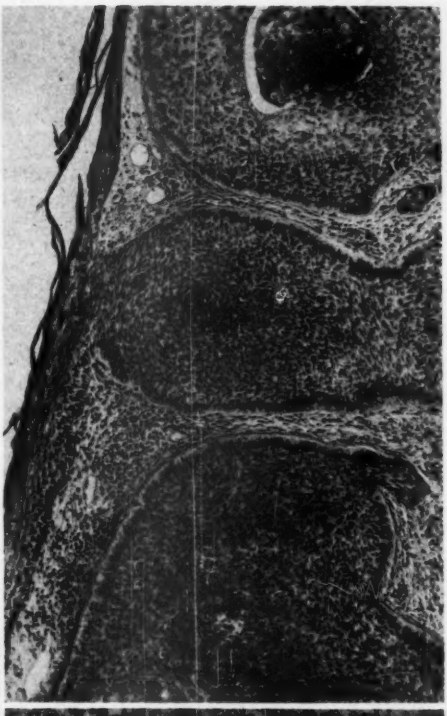
FIG. 2. Primordial type of adnexal carcinoma, its simplest form. For comparison with the diagrams in Figure 14. × 90.

FIG. 3. Cylindrical-celled or "ribbon" type, which does not betray its origin beyond presenting a slightly glandular appearance. × 90.

FIG. 4. Sudoriparous glandular type of the neoplasm. A long duct runs diagonally across the field; it may be a normal structure involved in the tumor or it may be of neoplastic origin. × 90.







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Foot

Adnexal Carcinoma of the Skin

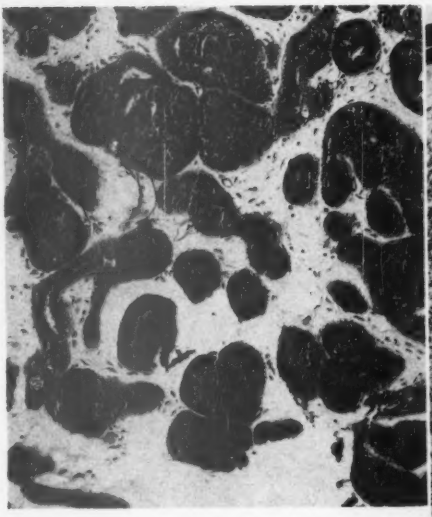
PLATE 2

FIG. 5. A typical solid hydradenoma for comparison. There are several ducts comprising two layers of cells, a particularly well developed one being apparent at the left of the field. × 80.

FIG. 6. Basal-celled type. There is some question whether this is not, in reality, a plexiform epidermoid carcinoma. × 80.

FIG. 7. Adnexal carcinoma impregnated with silver by the Nonidez-Ramon y Cajal method. All black filaments represent neurofibrillae. This technic does not impregnate reticulum to any extent. Of note are the small buds or bulbous terminals in the basement membrane of the complex at the upper right portion of the field. × 460.

FIG. 8. The earliest changes in the skin of the aural pinna in adnexal carcinoma. For comparison of the angular downgrowth with the diagrammatic primordium shown in Figure 16. × 60.



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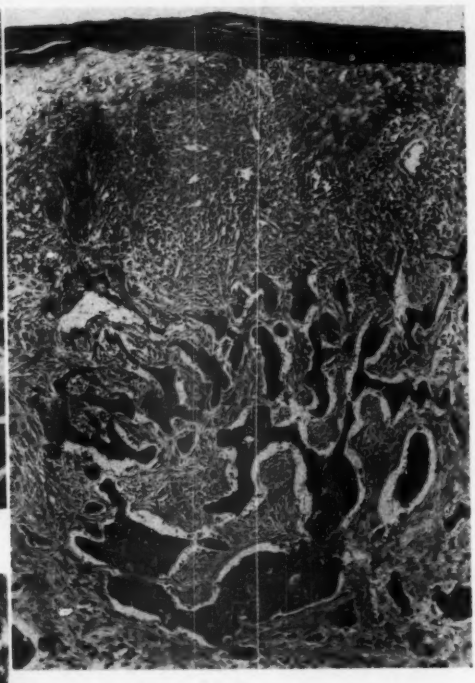

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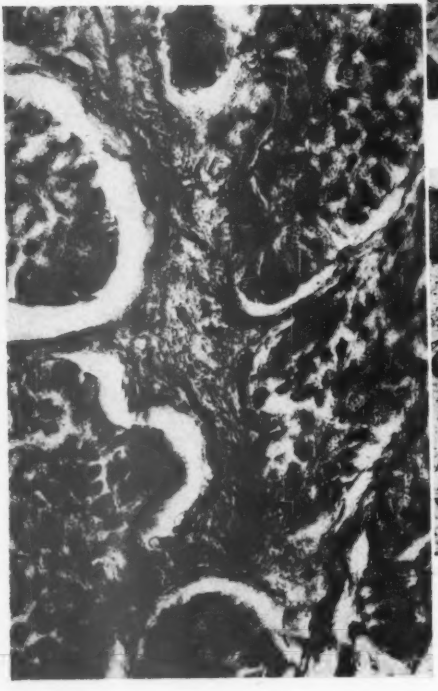

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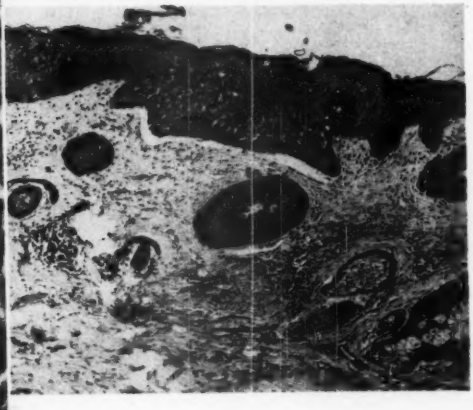

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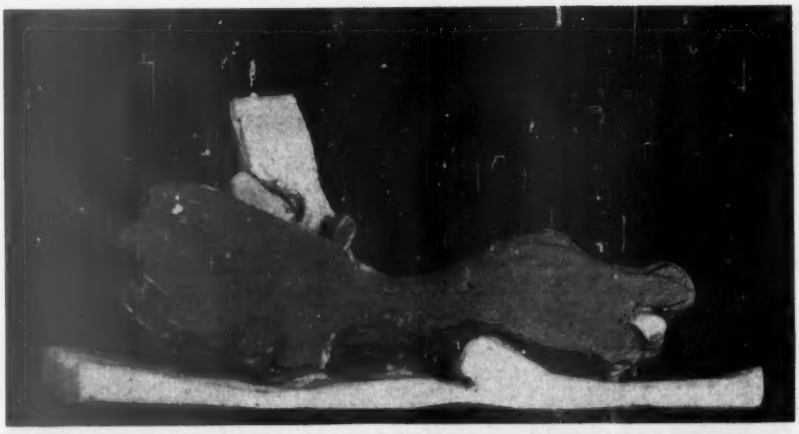
PLATE 3

FIG. 9. Reconstruction 1. Enlarged reproduction of a tuberous complex of adnexal carcinoma (gray) arising from a pedicle near the opening of a hair sheath (white, at top). Of note are the incipient sweat duct projecting near the hair sheath and the lobulated contours of the neoplastic mass. Model, × 70; photograph, about × 50.

FIG. 10. Reverse aspect of reconstruction 1. Two lobes of the tumor (gray) surround the neck of the hair sheath (white). The pedicle of the bilobed complex arises just behind the hollow channel of the hair sheath. About × 50 as photographed.

FIG. 11. Reconstruction 2 resembles the primordium of a sudoriparous gland. An abortive sudoriferous duct is seen projecting (in gray) above the hair sheath (white) at the right. × 70.

FIG. 12. Reverse side of reconstruction 2. All of the grayish portion is neoplastic tissue, the skin and a sebaceous gland are white. × 70.



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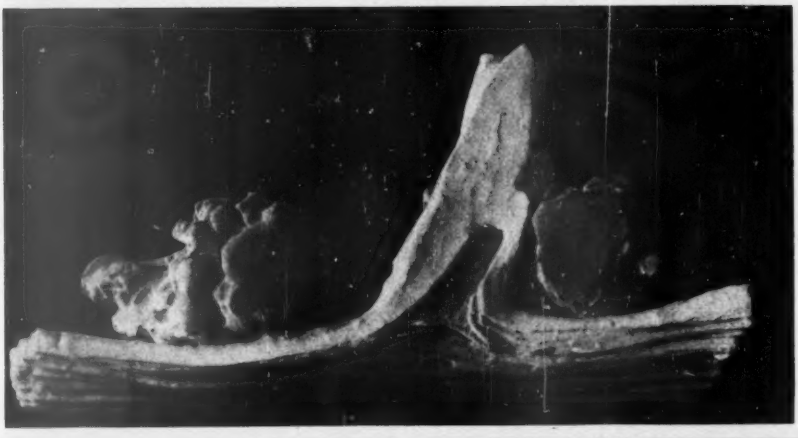

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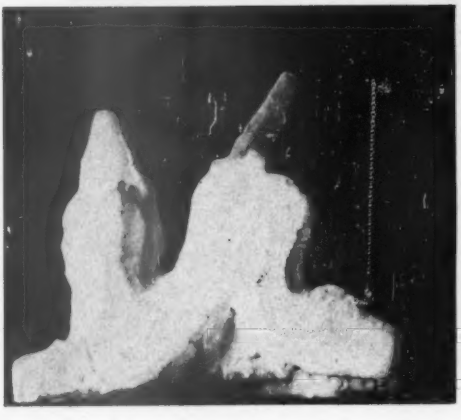

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Foot

Adnexal Carcinoma of the Skin

PLATE 4

FIG. 13. Sagittal microscopical section of the complex in reconstruction 2. Of note is a sudoriferous duct running down from the primordium-like mass. For comparison with Figure 16. × 95.

FIG. 14. Two early primordia of hairs from a 3-months-old embryo. No. 1 is the earliest beginning, no. 2 is slightly more advanced. "HK" is the embryonal hair matrix. (Fig. 304 of Maximow and Bloom, Textbook of Histology, 1931, ed. 1. Reproduced by permission of W. B. Saunders Company and Dr. William Bloom.)

FIG. 15. Two pilar primordia in more advanced stage of development. Of note are the accessory primordia at the necks of these embryonic follicles. For comparison with Figure 1. (Figure 305 of Maximow and Bloom, Textbook of Histology, 1931, ed. 1. Reproduced by permission of W. B. Saunders Company and Dr. William Bloom.)

FIG. 16. An eccrine sweat gland from the volar surface of the index finger. For comparison with reconstruction 2, Figures 11 and 12. (Figure 299 of Maximow and Bloom, Textbook of Histology, 1931, ed 1. Reproduced by permission of W. B. Saunders Company and Dr. William Bloom.)

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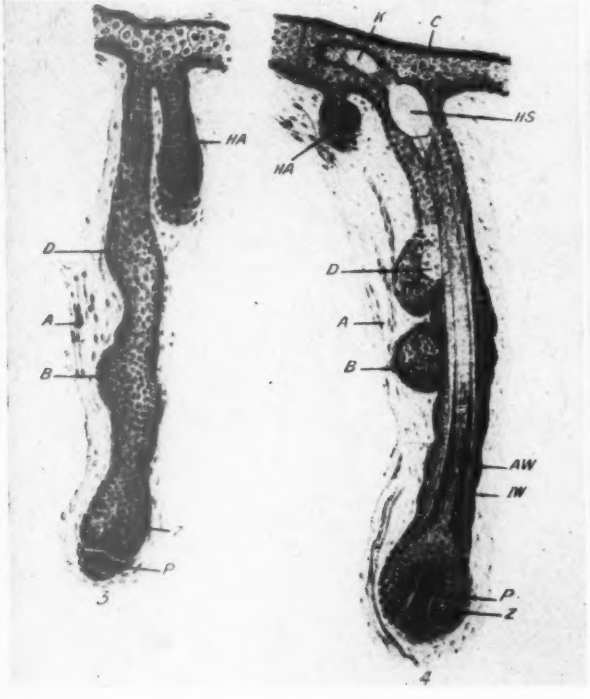

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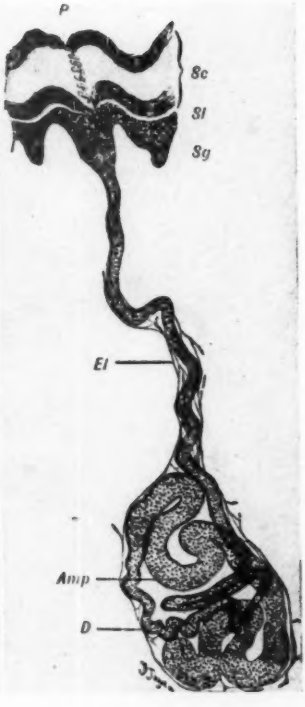

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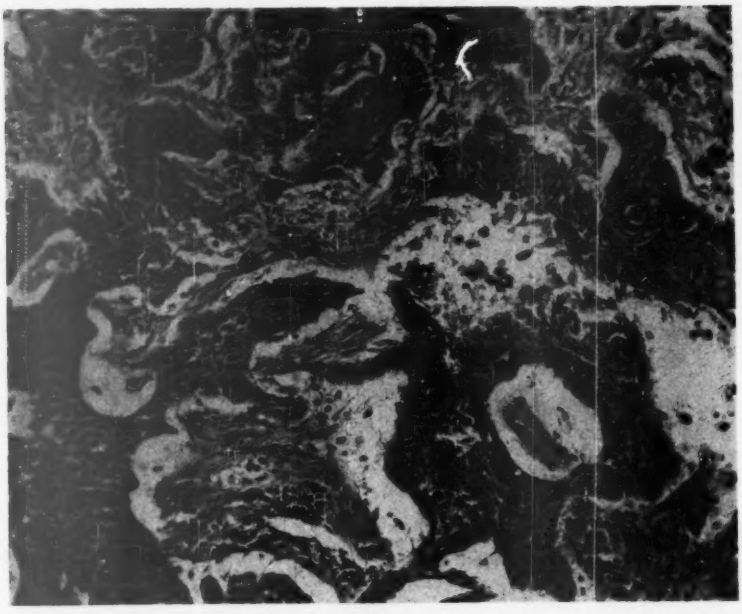
Adnexal Carcinoma of the Skin

PLATE 5

FIG. 17. A field from a metastasis of adnexal carcinoma in a lymph node in the subaural region. In it, differentiation toward pilar or sudoriparous structure is maintained in spite of the removal of the growth from its connection with skin. Some of the concentric structures contain keratinized débris; this might be abortive hair shaft material or keratinized lining of sudoriferous ducts.

FIG. 18. A sagittal section of the neoplastic complex in reconstruction 1 (one of a series from which it was built up). Of note is the very tenuous pedicle arising from the epidermis. The primordial type predominates. × 80.



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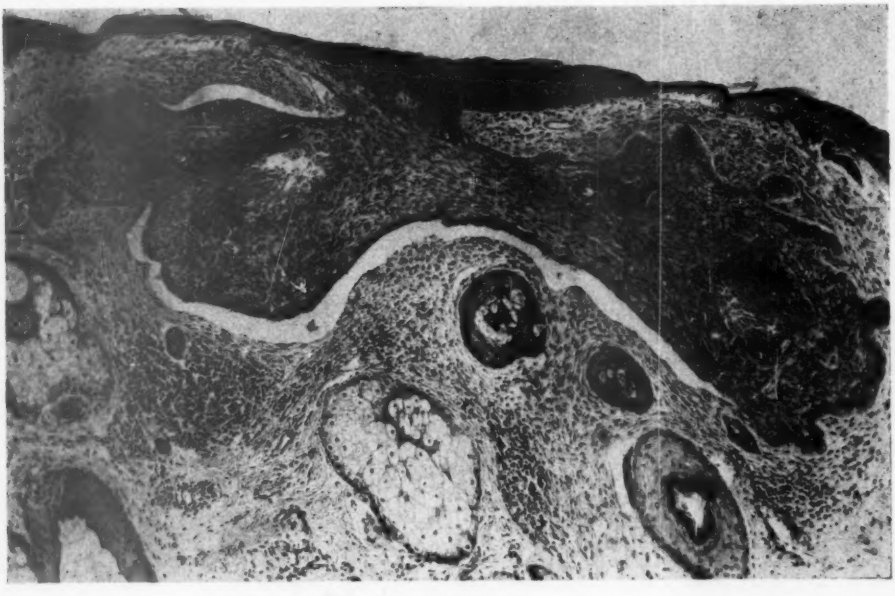

18

MUMMIFIED EPIDERMAL CYSTS

(So-Called "Calcified Epitheliomas")*

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The tumor known in the literature as "calcified epithelioma" is relatively infrequent. In 1933 Ch'in [1] reported 10 cases in a group of 22,000 consecutive surgical specimens, while in 1944 Highman and Ogden [2] described 12 instances from more than 24,000 specimens. Côté [3] discussed 12 examples in 1936, observed over a period of 8 years at Leeds General Infirmary. Fevre, Huguenin, and Paiz [4] found 9 cases in a period of 9 years at the Institut du Cancer in Paris, and discussed them in 1938. Sutton and Sutton [5] reported a single case, while other case reports have been described in the American and foreign literature [6-8] including multiple examples in a single patient. Gromiko [9] discussed a malignant transformation of one of these tumors. Warvi and Gates,[10] in their analysis of 566 cystic tumors of the skin, briefly mentioned 11 as calcified and one as ossified, but did not make it clear whether these conformed to the published reports of calcified epithelioma, or whether they represented simple calcification of epidermal cysts.

The nature of the tumor has provoked considerable difference of opinion. Most authors relate it to epidermoid cysts, "atheromas" of the skin, sebaceous cysts, and similar categories. It has also been considered a lesion *sui generis* as well as related to basal cell carcinomas. Warvi and Gates,[10] in their brief but penetrating discussion, enumerated different classes of tumors which may calcify, and which they subsumed under the generic term of calcified epithelioma.

It seems inescapable that more than one type of tumor has been so designated. Calcification may or may not occur. Cells may be squamous or basal in type. Viable epithelium may or may not be present. Most examples are clearly benign, but malignancy has been reported as well as benign recurrences. It is difficult to find any single characteristic which applies to all reported examples.

In my own material tumors of two distinct types have been encountered, each of which corresponds to some of the reported cases. It is the purpose of this communication to analyze these two types and trace their histogenesis.

My material is drawn from 7,500 consecutive surgical specimens.

* Received for publication, December 26, 1945.
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Nine cases have been selected, divisible into two categories. The first group, of 8 cases, includes 2 transitional examples. The ninth case is clearly different. All epidermal cysts in the files, to the number of 123, were also reviewed. The two transitional cases had been originally classified as epidermal cysts.

The patients, 8 men and one woman, ranged from 11 to 63 years of age. The tumors were located on scalp, face, neck, back, arm, and thigh. In duration the extremes were 3 months (of questionable accuracy), to 10 years. The gross pathologic features were not characteristic. The specimens varied in size from 0.8 to 3.5 cm. in greatest dimension. They were roughly and approximately encapsulated, with consistency varying from rubbery to hard. All specimens could be incised, but several required decalcification before sectioning. The exposed cut surfaces varied from grayish white to mottled tan, and from smooth to granular and crumbly. Small cystic spaces, 1 to 2 mm. across, were seen in 3 instances. For purposes of analysis, brief individual microscopic descriptions are given.

Microscopic Descriptions

Case 1. In the lower corium was a well circumscribed, encapsulated tumor consisting of small masses of epithelial nature, separated by bundles of collagen. The epithelial masses were entirely nonviable, and were composed of ghost-like shadows and outlines. They appeared to have been originally squamous in character. Distinct cytoplasmic outlines were visible, but no intercellular bridges were seen. Most cells showed a faint nuclear shadow. There were extensive deposits of deep-blue-staining material, identifiable as calcium, which encrusted the nuclei and was present as a fine dusting within the cytoplasm. The stroma intervening between the cell masses was very poorly vascularized. A few multinucleated foreign body giant cells with very well stained nuclei were seen, in contrast to the mummified epithelium. No bone formation was present. Figure 1 illustrates this tumor. The mummified epithelial masses with distinct cell outlines show heavy deposition of calcium granules around the periphery, with a sparser dusting in the central portions. Shadowy outlines of epithelial nuclei can still be seen. The poorly cellular and poorly vascularized stroma is composed of dense collagen.

Case 2 was similar to case 1 in all essential respects.

Case 3. The tumor of case 3, generically similar to the preceding, showed a few noteworthy differences. The masses of mummified epithelium, with distinct cell boundaries, were much larger, and there was a corresponding diminution in the amount of fibrous stroma. Abundant

calcification was present, proved by the von Kossa stain. But in addition, extensive amounts of iron-containing pigment were observed, confirmed by the Prussian blue reaction. This pigment was seen as a fine dusting of epithelial cytoplasm and nuclei, and in large masses within the stroma. Iron and calcium co-existed in the same cell groups.

Case 4 was of unusual interest, since the pathogenesis of the tumor could be clearly traced. The patient was a soldier, 38 years old, who had suffered multiple battle wounds. One shrapnel fragment struck him in the forehead. The wound drained for about 2 months and then a tumor developed over the next 6 months. Histologically, the picture was very similar to that of cases 1 and 2, except that there was a vigorous round-celled infiltration in the stroma, with large numbers of foreign body giant cells. Calcification was much less intense. Figure 10 illustrates this case. In one portion of the tumor there was a small, typical epidermal cyst with a narrow peripheral rim of viable squamous epithelium, and a content of partly calcified, fused, keratinized material, in which cell outlines were not visible.

Case 5. The tissue of case 5 consisted of relatively large masses of mummified epithelium, showing variable amounts of calcification encrusting both nuclei and cytoplasm. The squamous character of the epithelium was clearly visible, but the staining reactions did not indicate viability. In parts of the specimen the mummified cell masses were closely invested by well formed bony trabeculae with clearly staining bone corpuscles and many scattered cement lines. There was no osteoblastic activity. Elsewhere there was the usual fibrous stroma. Figure 2 illustrates the excellently formed bony trabeculae, with marrow spaces. The bone is in immediate apposition to the nonviable epithelium, the cell boundaries of which are clearly visible.

Case 6. Pathologically, case 6 was virtually identical with case 5.

It is clear that these 6 cases are all essentially similar. The presence or absence of bone formation in the stroma is of no fundamental significance. It must be interpreted as a metaplasia of the stroma, present in some instances, absent in others, without affecting the fundamental nature of the tumor. Similarly, the very large amount of iron in addition to the calcium in case 3 may be considered an indication of unusual local metabolic factors, in a case otherwise similar.

The traumatic origin of case 4 distinguishes it from the others in this series which arose spontaneously. That epidermal cysts or implants may arise from local trauma, often extremely slight, is universally recognized. That a typical example of "calcified epithelioma" should arise from definite trauma strongly suggests the kinship of this lesion with the epidermal cyst.

Discussion of Simple Epidermal Cysts

Before considering the transitional cases, simple epidermal cysts may be considered. In a series of such cysts certain definite gradations can be observed. As the basic type may be designated the familiar form in which there is a narrow intact lining of squamous epithelium with well defined basal and prickle layers and a definite, although narrow, keratohyaline layer. The cyst contents consist of flaky, loosely lamellated keratotic material (Fig. 5). Sometimes this material is inspissated, fused, and partly hyalinized. In routine stains the flaky squames are tinged a faint blue or lavender, while the dense material is pink. Occasionally, faint traces of nuclei are seen in the cyst contents, but cytoplasmic outlines and membranes are lost.

The lining epithelium is not always regular and even. Occasional areas of proliferation are observed, in which conical, bud-like, papillary, or trabecular processes extend inward (Figs. 6 and 7). The epithelium, instead of being entirely orderly and reproducing the structure of the normal epidermis, may exhibit dyskeratosis. Instead of a definite, flattened, keratohyaline layer, the marginal cells may be enlarged, rounded, and pale-staining, with frequent perinuclear vacuoles, and alterations of the size and staining qualities of the nuclei. Such pale, ballooned cells are seen in Figures 6 and 9. The cyst contents may then reveal certain differences from the more usual type. Instead of consisting of loose or dense, simple, keratinized material, the contents may exhibit parakeratosis (Fig. 6) with persistence of nuclei which are flattened and pyknotic. There is also a tendency towards persistence of cell outlines and cell membranes. Epithelial whorls, of shadowy character, may be present in the center of cysts, surrounded by inspissated, fused, keratinized material. In these whorls cell outlines are clearly preserved, and the plump cell contours contrast with the surrounding, fused keratin.

These variations in cyst contents, as compared with Figure 5, are apparently related to the character of the lining epithelium. Whorls, persisting nuclei, and preservation of cell contours and membranes are seen where the lining epithelium shows disorder of architecture, alterations of cell structure, and departure from the normal epidermal pattern. To such changes the term dyskeratosis * may be applied, relative to the viable epithelium forming the rim of an epidermal cyst.

The contents of epidermal cysts are prone to calcification. I have

* This use of the term differs from that employed by Highman and Ogden [2] in their discussion of "calcified epithelioma." They apparently applied it to nonviable degenerated cells of the type that I have called "mummified cell masses." In my opinion the term should apply only to viable epithelium showing disorders of cellular structure and organization. There is no necessary implication of carcinoma.

never observed calcification when the contained material is of the loose lamellated type (Fig. 5), but only when it is dense and inspissated. Calcification of the contents is independent of the degree of regularity or of dyskeratosis of the lining epithelium.

Another very frequent complication is rupture and destruction of the epithelial rim, with attendant granulomatous reaction. Leukocytes and foamy macrophages vary in number. In one instance actual bone formation was observed in the granulation tissue bordering such a cyst. The epithelial lining in this instance was completely destroyed, but occasionally persisting partial arcs of epithelium lay adjacent to granulomatous or fibrous areas.

These variations in structure of epidermal cysts are of importance in considering the histogenesis of so-called calcified epithelioma.

TRANSITIONAL CASES

Case 7. Around the greater part of the periphery of the cyst in case 7 there was a delicate fibrous capsule, within which was a layer of squamous epithelium showing a few minute papillary buds. At the inner free margin the epithelial cells were large, pale, granular, and obviously degenerating (Fig. 9). Many of these nuclei were shrunken or pale-staining. The central portion of the mass was composed largely of keratinized material, staining dull red, in which cytoplasmic outlines were excellently preserved (Fig. 8). Nuclear outlines were ghostly and shadowy. Numerous large masses of calcium salts were present. At one edge the epithelial lining was incomplete and there was invasion by granulation tissue containing many large macrophages, a few of which were lipid-bearing. This granulation tissue, affecting only a small part of the specimen, penetrated among the masses of keratotic material, breaking it up into smaller irregular masses. Polymorphonuclear leukocytes were conspicuously absent.

Case 8. The specimen from case 8 was a small cyst from the upper eyelid, present for 3 years. It was essentially similar to that of case 7 except for its smaller size. It presented in excellent fashion the incomplete layer of lining squamous epithelium which, away from the basal layer, revealed marked dyskeratosis. The center of the cyst was filled with masses of mummified material similar to those shown in Figure 10. Although the lining epithelium was not complete, there was no significant invasion of the cyst content by connective tissue.

The specimens from cases 7 and 8 were originally diagnosed as epidermal cysts with calcification. Only after the series of typical calcified epitheliomas was examined and the epidermal cysts re-studied, was their transitional character appreciated. The significant features were:

(1) a partial layer of squamous epithelium around the periphery, characteristic of epidermal cysts, but showing definite dyskeratosis; (2) cyst contents composed of cornified epithelial cells which presented an unusual degree of preservation of the cytoplasmic outlines; (3) calcification within the cyst contents; (4) rupture of the epithelial lining, with granulation tissue invasion, breaking up the cornified masses into smaller aggregates separated by fibrous tissue.

It is easy to imagine the probable future progression of cases 7 and 8 had there been no surgical intervention. The connective tissue invasion would have progressed to involve the entire contents, breaking it up into irregular smaller masses; the peripheral rim of viable epithelium would disappear; more calcium would be laid down within the keratinized or mummified epithelial masses; the invading connective tissue stroma would become less cellular in time; ossification might or might not develop. If the features already present in cases 7 and 8 progressed in this fashion, there would result in time a picture exactly like that of case 1 or case 5. Yet there can be no doubt that cases 7 and 8 represent epidermal cysts, and can be classified with the calcified epitheliomas only as early and transitional examples.

Case 9. The final example, a mass in the anterior triangle of the neck of 1 year's duration, presented a very different picture from any of the preceding cases. It was classified with the calcified epitheliomas in the literature, but should be definitely separated from the preceding group.

In this tumor there were broad anastomosing ribbons of cells of basal type, forming a convoluted pattern and composed of very densely packed viable nuclei, with numerous mitotic figures. The nuclei were moderately chromatin-rich, with a single nucleolus. No cytoplasmic differentiation was apparent, but the nuclei appeared embedded within a vague symplasmic mass. Figure 3 illustrates this tumor. The significant feature was the presence of squamous cells at some of the margins of these cellular ribbons. The interstices between some of the ribbons were, in turn, filled with masses of mummified squamous epithelium, which appeared to be in relation to the living squamous epithelium. Figure 4 illustrates this feature. In this figure the densely crowded nuclei, clearly of the basal cell type, appear to be those of living cells. Small areas of squamous epithelium are apparent as well as larger masses of mummified cells.

Discussion

It is my contention that the fundamental property of so-called calcified epithelioma is the presence of well preserved masses of squamous-appearing cells, completely non-living, and generally with faint

outlines of nuclear structure. The presence of actual calcification in such masses is not essential, nor is the occurrence of viable epithelium. The question then arises, what is the origin of these mummified masses? The opinion is expressed in the literature that they represent either necrotic or "dyskeratotic" epithelium. It appears from the transitional cases and simple epidermal cysts described herein that, on the contrary, this material is a cornification product, desquamated from a layer of viable squamous epithelium. In the course of the progression, the viable epithelium may disappear to leave only the resistant cornified material. This may or may not become calcified.

In support of this view, the following data may be emphasized. A squamous epithelial layer, especially when lining a cyst but also when covering a free surface, may give rise to several different types of product. A flaky atheromatous material, a definite parakeratosis, a fused and hyaline mass without cell outlines or nuclei, and a cornified tissue preserving the cell and nuclear outlines, all may be seen in different lesions, especially in contents of cysts. Such changes may also be seen in the horny layer of some verrucae vulgares. In these tumors, as in epidermal cysts, several different types of cornified material may be produced consecutively by the same germinative layer. Especially in cyst linings, the still living cells may exhibit degenerative and regressive changes and morphologic alterations which may properly be called dyskeratotic. These changes, apparently an expression of altered growth potencies, may well be precursors of the changes here called mummification.

I have adopted the term "mummified" from the French "momifié," which appears to express most accurately and picturesquely the appearance of the cells. The outlines are preserved. The changes within the cytoplasm are not known, but they are profoundly different from the alterations attending other types of cornification. This is apparent from the different tissue reactions invoked by various types of epidermal cyst contents when connective tissue invasion occurs. Sometimes abundant lipophagic reaction is seen. Lymphocytes, macrophages, polymorphonuclear leukocytes, and foreign body giant cells may be seen in varying proportions. Study of epidermal cysts, as well as of transitional cases 7 and 8, strongly suggests that the various fused and hyaline types of keratinization are quite susceptible to lysis, digestion, and connective tissue invasion. On the other hand, the mummified cell groups seem peculiarly resistant. Although foreign body giant cell reaction is common, no significant lysis is observed. The onset of calcification renders absorption even more difficult, and the resulting lesion may be considered analogous to the persistence of a lithopedion.

In the production of the fully developed quiescent lesion, as in cases 1, 2, 3, 5, and 6, the histogenesis may be as follows. Epidermal cyst contents of varying types, following disappearance of the viable peripheral cell layer, are invaded by organizing connective tissue. The cornified but nonmummified material is absorbed and replaced by connective tissue, but the mummified masses, being resistant to lysis, remain relatively unaltered, and finally calcify. As an alternative, the lining of epidermal cysts may proliferate inward, as seen in Figure 7. Such convoluted epithelial ribbons can become dyskeratotic and give rise to cornified material. If the viable epithelium disappears, as seen so commonly in epidermal cysts, the mummified masses may be very irregularly distributed after connective tissue invasion has replaced the other elements.

Case 9 seems to be a lesion of quite different character. The closely packed viable nuclei of basal cell character strongly suggest a basal type carcinoma. Some portions, however, reveal definite squamous differentiation of almost epidermal character. It seems probable that cells of this latter type give rise to the mummified tissue illustrated in Figure 4. The presence of such material is the sole, but adequate, justification for classifying this case with the others. The lesions described in the literature as recurring are all of this type.

In reference to nomenclature, Côté [3] has pointed out that the term epithelioma, applied to these lesions, is taken from the French, in which language it connotes simply an epithelial growth. The English usage of epithelioma, on the other hand, connotes cancer. That "calcified epithelioma" is a misnomer is generally recognized, but since recurrences and even malignant proliferation have been reported, the term has persisted. In view of the histogenesis expounded in this paper, it is suggested that for the first type of lesion (cases 1 to 8) the term "mummified epidermal cyst" be adopted. Calcification, as well as ossification of the stroma, are accidental features which do not affect the essential nature of the lesion.

Case 9 and the similar cases reported in the literature seem to be of a different category, and may properly be called "basal carcinoma with squamous differentiation and mummification," or, more simply, "basal carcinoma with mummification."

Conclusions

The term "calcified epithelioma," as used in the literature, has been applied to two clearly separable types of lesions having one feature in common, namely, masses of nonviable squamous-appearing cells of mummified appearance. These mummified masses show clearly de-

fined cell membranes and faint nuclear shadows. There may or may not be deposition of calcium granules, and rarely iron pigment may also be seen. In the first type of "calcified epithelioma," the mummified material is present within a fibrous stroma which may show metaplastic ossification. This type may be traced to simple epidermal cysts through successive steps of dyskeratosis of the lining epithelium, production of cornified material preserving cell outlines and contrasting sharply with the usual contents of epidermal cysts, disruption and disappearance of the epithelial layer and invasion of cyst contents by granulation tissue, resistance of the peculiarly cornified (mummified) material to lysis, and its persistence in the face of vigorous organization elsewhere. Calcification enhances the permanence of the mummified material.

The second type of lesion shows characteristics of basal cell carcinoma, with areas of squamous differentiation and production of mummified keratinized material. Such lesions may recur if operative removal is incomplete.

The term "calcified epithelioma" is a complete misnomer and should be abandoned. For the first type of lesion the name "mummified epidermal cyst" is suggested; for the second, "basal carcinoma with mummification."

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 6

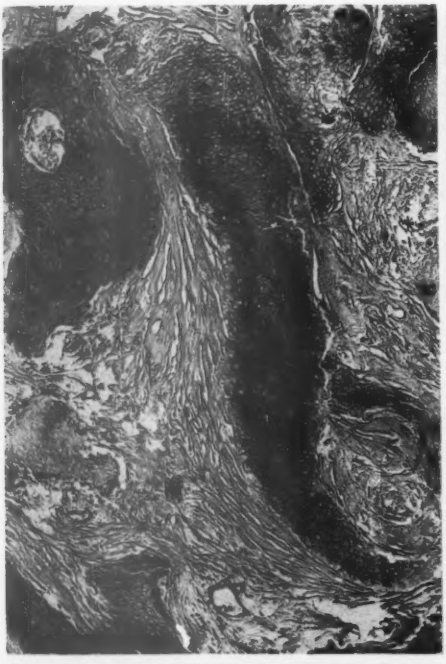
FIG. 1. "Calcified epithelioma" (case 1) showing masses of mummified squamous cells with persistence of nuclear shadows, and abundant calcification at the periphery of the masses. The stroma is dense hyaline connective tissue. × 125.

FIG. 2. "Calcified epithelioma" (case 5). The mummified cell masses are invested by a stroma with well formed bone. × 200.

FIG. 3. Basal cell type of "calcified epithelioma" (case 9). The nuclei all stain well with hematoxylin and are entirely viable. × 200.

FIG. 4. Another view of case 9, showing basal type of cell in lower right corner, squamous differentiation in upper right, and mummified squamous material on left. × 500.

1



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3

4

Plate 7

Fig. 5. Epidermal cyst, with flaky atheromatous type of contents. × 250.

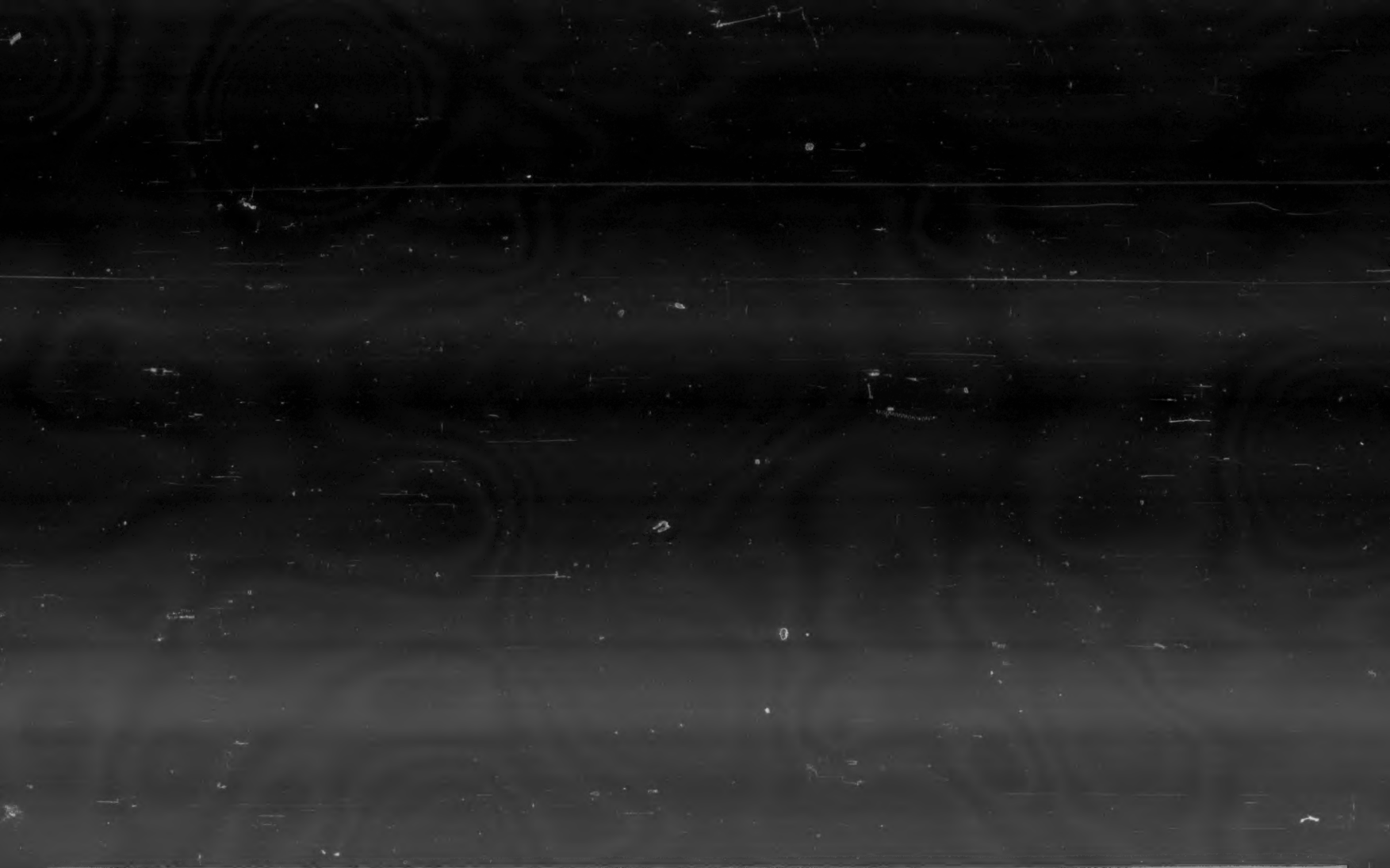
Fig. 6. Epidermal cyst, with papillary type of epithelial lining. The cyst contents exhibit some parakeratotic elements mingled with well preserved cell outlines and inspissated cornified material. The epithelial layer shows dyskeratosis. × 200.

Fig. 7. Epidermal cyst with trabeculated and proliferated epithelial lining. The contents show hyalinized and inspissated material. × 200.

Fig. 8. Case 7, showing cyst contents. Part consists of well cornified cells with preserved outlines and persisting nuclear shadows, forming a mummified tissue. There is a sharp line of demarcation from the granular amorphous contents, presumably representing lysed débris. × 500.

Fig. 9. Case 7, epithelial lining, with dyskeratosis, and mummified desquamation products. × 500.

Fig. 10. Case 4. Mummified material with foreign body reaction. The larger masses are separated by granular débris. (For comparison with Fig. 8.) × 400.



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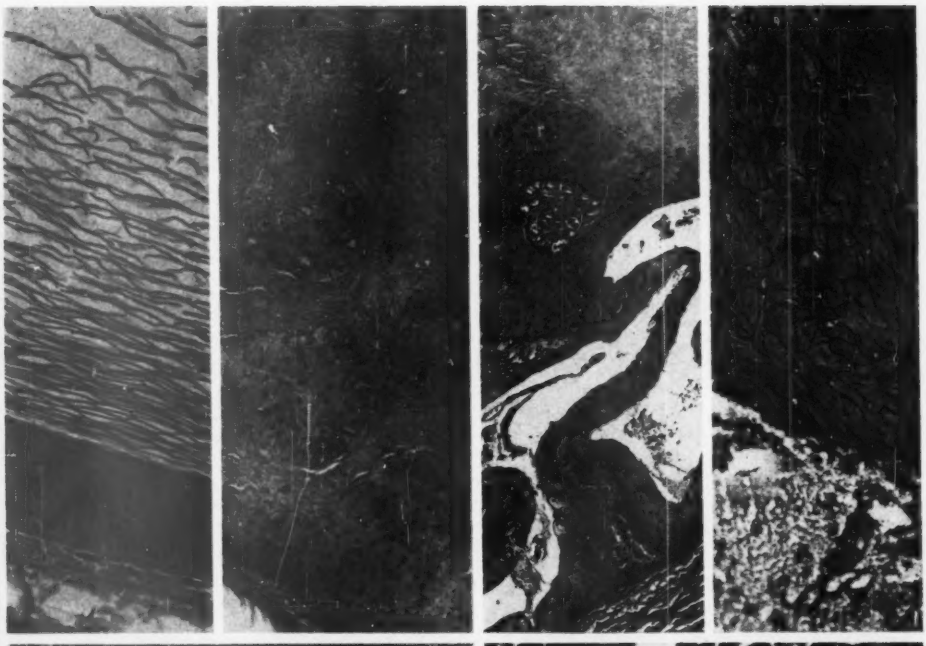

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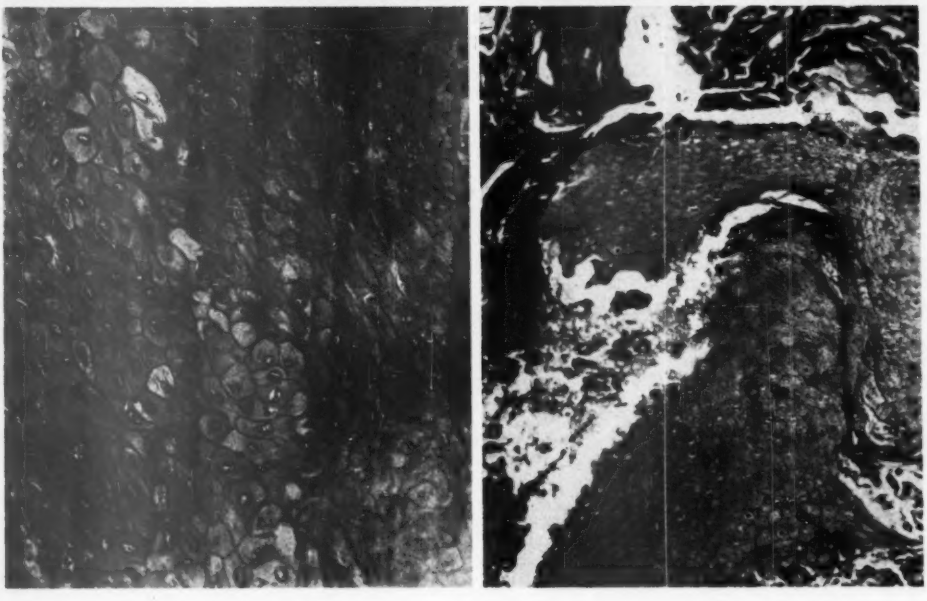

EWING'S SARCOMA OF BONE *

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When one studies Ewing's reports of 1921,[1] 1924,[2] and 1928,[3] it is clear that he was attempting to single out among the primary malignant tumors of bone an entity which he first called "diffuse endothelioma" and subsequently "endothelial myeloma" of bone. He thought that the tumor cells were derived from angio-endothelium in the broadest sense. He described the type cell as a small polyhedral cell with pale cytoplasm, small hyperchromatic nucleus, and well defined cell border, and stressed the idea that the tumor cells showed no osteogenic potentialities. He stated that, although tending to be arranged in compact broad sheets, the tumor cells, at least in some places in a given tumor, were often found lying around tiny or larger vascular spaces in "perithelial" arrangement or still circularly, but not around a vessel, in "rosette" formation.

Ewing also consistently stressed certain clinical features as likewise being important in the delimitation of the bone tumor he was discussing. In his opinion, these included: youthfulness of the patients (on the whole); a rather characteristic roentgenographic appearance of the presenting bone lesion; a gratifying initial response of this lesion to radiation therapy; and eventually (in all but a few cases) the appearance of lesions in other bones and especially in the lungs, with fatal result.

However, the concept of Ewing's sarcoma as delineated above has undergone certain criticisms and modifications. Some investigators have even doubted the validity of the basic concept itself, pointing out that it had been founded on, and largely sustained by, clinical and biopsy findings, rather than by study of cases followed through to the end and systematically autopsied. In relation to diagnosis, so much faith has long reposed in the clinical aspects, as sketched above, that, summarized as constituting "Ewing's syndrome," they often took precedence over tissue examination in arriving at a diagnosis—a point of view which has proved itself unjustified. Many, including Neely and Rogers,[4] Swenson,[5] and Barden,[6] have pointed out that evidence is lacking that the roentgenographic picture is sufficiently characteristic for the condition to be of high diagnostic value. Colville and Willis [7] and Willis [8] also emphasized the pitfalls involved in arriving at a diagnosis even through biopsy, and the diagnostic surprise frequently encountered when a case suspected of being Ewing's sarcoma is finally subjected to detailed post-mortem examination.

* Received for publication, January 28, 1946.

With due allowance for all these reasons for diagnostic caution, the weight of evidence sustains the existence as an entity of a primary bone sarcoma to which, because of Ewing's pioneer efforts to single it out, the name of Ewing's sarcoma of bone can justifiably be applied. Yet, without prejudice to that central fact, it should be noted that Oberling [9] and Oberling and Raileanu,[10] among others, have dissented from Ewing on the histogenesis of the tumor cells, as well as on the details of the cytology of the tumor. As to histology, Oberling found that the cells of the neoplastic tissue proper tend to appear in a sort of network in which nuclei are prominent, cell cytoplasm is meager, delimiting cell membranes are lacking, and the cells are connected by short or long cytoplasmic processes. He further described the nuclei as uniform in size and generally roundish or ovoid, and as having powdery chromatin and often one or more nucleoli.

On the basis of our own material (17 cases, 4 of which were autopsied) we found that, provided the neoplastic tissue was definitely viable and well fixed and well stained, and irrespective of whether it came from the presenting bone lesion or from a metastatic focus, the appearance of the basic tumor cells corresponded rather to the description given by Oberling than to that given by Ewing. Also, we could not convince ourselves that perivascular orientation of the tumor cells was a characteristic cytologic feature of the tumor in question, although we found that tumor areas which had been heavily invaded by blood vessels, especially in the wake of hemorrhage, often showed tumor cells about capillary spaces or around larger vascular spaces in so-called "perithelial" arrangement. Furthermore, our material failed to provide evidence of the presence of the true rosette (or pseudorosette) formations which have been stressed as part of the cytologic picture of Ewing's sarcoma.

As to histogenesis, Oberling maintained that the tumor develops from the immature reticular cells (the supporting mesenchymal cells) of the bone marrow. For this reason, he conceives and designates Ewing's sarcoma as a reticulosarcoma of bone marrow, and also believes in the kinship (falling short of identity) between reticulosarcoma of bone marrow and the generally recognized reticulosarcoma of lymph nodes. Also, basing his idea on the view that the reticular cells of the bone marrow are totipotent, he maintains that, in some areas of a reticulosarcoma of bone marrow (Ewing's sarcoma), these cells may differentiate in the direction of endothelial cells (cells capable of forming vascular and lymphatic channels) or even in the direction of hemocytoblasts (cells capable of forming myeloid or lymphoid cells). Ewing, taking cognizance of Oberling's views, rejected this conception of the histogenesis of the tumor. Instead, he reiterated, both in 1939 [11] and

in 1940,[12] that the tumor denoted by his name must be conceived as arising from cells which are strictly of angioblastic nature and possess no wider potentialities than the formation of vascular channels.

Like Stout,[13] we saw no evidence in our material that the basic cell of the Ewing sarcoma is totipotent, as Oberling believes, and able to differentiate in the direction of cells capable of forming vascular and lymphatic channels, or even in the direction of cells capable of forming myeloid or lymphoid cells. Like Stout, too, however, we favor Oberling's interpretation of the tumor as a sarcoma of a primitive form of connective tissue and specifically of the mesenchymal supporting framework of the bone marrow. Nevertheless, we feel that designating the lesion as a reticulosarcoma is open to misunderstanding because of the confusingly varied interpretations existing as to the neoplastic potentialities of the mesenchymal reticular framework of the lymphoid and myeloid tissue. Also, it should be pointed out that Parker and Jackson [14] described, under the name of "primary cell sarcoma of bone," a tumor which they hold akin to reticulum cell sarcoma of lymph nodes but distinct from Ewing's sarcoma. For these reasons, and because one might also be thought to believe with Connor [15] that it should be classed as a tumor of the reticulo-endothelial system, we have preferred to use the neutral name of "Ewing's sarcoma" instead of "reticulosarcoma" for the tumor in question.

Clinical Features

Age and Sex Incidence and Localization

At the time of admission to the hospital, the 17 patients in our series ranged between 4 and 39 years. However, all but 4 of them were 12 to 19 years of age (these 4 being 4, 8, 24, and 39 years). Correspondingly, the median and average age of the group as a whole were about 16 and 17 years, respectively. It may be that the strikingly narrow age range in our series is favored by the small number of cases, but at any rate our data are in line with the general observation that a very large proportion of the cases in any series (see, for instance, Hamilton [16]) fall within the second decade of life.

Eleven patients (65 per cent) were males, which is in agreement with the experience of most observers that the disorder is slightly more common in males than in females. At any rate, the sex difference in incidence of the disorder is not striking.

Usually, only one skeletal lesion was causing complaint and was demonstrable roentgenographically at the time of the patient's admission to the hospital. In an occasional case, even on admission roentgenographic examination of the rest of the skeleton revealed one or more

additional but clinically silent foci of bone involvement or even pulmonary metastases. However, in connection with localization, we shall refer only to the presenting lesion—the one giving rise to the complaints which brought the patient to the hospital.

In 12 of our 17 cases, the presenting lesion was in one of the bones of the trunk. Specifically, this bone was an ilium in 4 cases, an ischium in 2, a pubis in 1, a rib in 2, a scapula in 1, a clavicle in 1, and a vertebra in 1. In the remaining 5 cases, the presenting lesion was in a long bone, and, specifically, it was in a humerus in 2 cases, a femur in 2, and a fibula in 1. Why, in our cases, the presenting lesions were preponderantly in bones of the trunk we do not know, but this has been true, also, in the experience of others.

History of Trauma

In only 5 of our cases was there a history of definite, fairly recent trauma to the site in which the presenting tumor developed, the trauma having antedated the discovery of the tumor by 1 to 6 months. As to the other cases, 1 patient stated that he had twisted his ankle 4 years before admission for a tumor in the fibula, but the actual complaints which had brought him to the hospital were of only 1 year's standing. Two patients implicated functional trauma of very recent occurrence: handball playing by one and broad-jumping by the other. In 2 additional cases, the site of the trauma mentioned by the patient was near, but not the same as, the site of the presenting tumor. In the remaining 7 cases, the patients gave no history of antecedent trauma to the region of the presenting lesion.

From these data, it is difficult to draw any conclusion as to a possible causal relation of trauma to Ewing's sarcoma. The 5 cases in which a definite history of possibly relevant antecedent trauma was recorded are counterbalanced by the 7 in which there was no history of such trauma. In the remaining 5 cases, the weight of the evidence is against the significance of the trauma, since the trauma was merely a mild functional one, or was not at the site of the subsequent tumor, or preceded the appearance of the tumor by an implausibly short period. Certainly, the data constitute no overwhelming evidence in favor of a causal relation between trauma and Ewing's sarcoma.

Clinical Complaints and Findings

Survey of the clinical histories of the patients in our series shows that local (and/or referred) pain was the one consistent complaint. With few exceptions, the pain was of at least some months' standing, and in several cases it had been present for at least 1 year before admission. Usually, also, it had become increasingly severe and persistent

during some weeks or months immediately before admission. With the local pain, there were often complaints related to spread of the tumor beyond the limits of the bone and varying with the location of the presenting lesion. Thus, for instance, from patients in whom some part of an innominate bone was involved, there were usually complaints of disability relating to the hip joint and sometimes also of radiating pain down the lower limb. In connection with presenting lesions near the end of a long bone, there were sometimes complaints of lameness or stiffness of the corresponding joint, and, in one case in which the lesion was near the lower end of the femur, there were repeated serous effusions into the knee joint. In the cases in which the presenting lesion was in a lumbar vertebra, there were, in addition to the local pain, complaints ascribable to implication of nerve trunks in the area, such as pain radiating down the limbs, and tingling sensations and weakness in the latter. Location of the presenting lesion in a rib was found associated with pleural effusion in one case. Other locations of the presenting lesion (for instance, in the skull) are associated with their own special clinical disabilities.

Just as local pain was the dominant clinical complaint, so the presence of a local tumor mass was the dominant clinical finding at the time of admission. On admission, a more or less prominent tumor mass was palpable at the site of the presenting bone lesion in all but 3 of our 17 cases. This fact indicates the strong tendency of Ewing's sarcoma to break out through the cortex of the bone and spread in the surrounding tissues. Notably large tumor masses were palpable in some cases in which the tumor appeared in an innominate bone. Spreading internally toward the pelvic cavity, the tumor beyond the limits of the bone could then sometimes be palpated as an elastic, irregular, globular mass, through the rectum if the tumor was low down, or in the lower quadrant of the abdomen if it was higher up. Spreading externally, a tumor springing from an innominate bone sometimes produced a large tumor mass palpable in the groin or in the gluteal region. In one of our cases in which the presenting lesion was in the shaft of a humerus there was likewise a very large extra-osseous tumor mass connected with the bone. When the presenting tumor was in a superficially located bone such as a clavicle or a rib, the mass produced by extra-osseous spread could be seen as well as palpated.

Tenderness to pressure at the site of the lesion was recorded in practically all cases. Frequently, the subcutaneous veins overlying the presenting lesion were found to be prominent. However, it was only exceptionally that increased local heat was mentioned in connection with the physical examination.

A survey of the temperature charts and the laboratory findings in

our cases revealed what appeared to be significant information of clinical value. Many of the patients were in the hospital for almost a week before specimens were secured for biopsy. During this time they had a slight fever, with daily rises in temperature to about 101° F.* These patients generally presented a secondary anemia (with a red blood cell count of about 3,500,000), and sometimes also a leukocytosis. In addition, they usually showed a high sedimentation rate of the blood. Taken together, these findings proved to be more significant in respect to the immediate prognosis than the size of the presenting lesion. Specifically, the cases in which some fever and secondary anemia, together or even alone, were noted ran a fulminating course, ending in death within a few months after admission to the hospital. On the other hand, those patients who had no fever on admission, and no anemia or increased sedimentation rate, tended to survive for a year or more after admission.

Roentgenographic Features

Roentgenographic Appearance of the Presenting Bone Lesion

By the presenting bone lesion we mean, as already indicated, the one causing the complaints which led the patient to enter our hospital. This was often the only lesion discernible even when the entire skeleton was roentgenographed on admission, and in any event it was the one which guided roentgenographic diagnosis. One need only review the presenting lesion in a series of cases to appreciate the difficulty of making a diagnosis of Ewing's sarcoma by x-ray examination alone. If the amount of bone involvement in the presenting lesion roentgenographically is still small and no lesions are found elsewhere, the picture may be misconstrued as an inflammatory lesion. However, in most cases the picture of the presenting lesion suggests a malignant tumor, although often misinterpreted as some malignant tumor other than Ewing's sarcoma.

* One case presented a diagnostic problem not only because of a definitely febrile course but because of atypicalness of the local clinical complaints and findings. The patient was admitted to the hospital complaining of difficulty with the right knee joint, dating back 8 months. The knee was painful, warm, tender, and swollen from the presence of fluid in it. Some rarefaction of the cortex and spongiosa of the medial condyle of the femur was noted roentgenographically, but there was no clear-cut indication of the presence of a tumor in the bone or overlying soft parts. The clinical impression was that the condition of the knee joint proper and of the condyle had its basis in some infection, as indicated by the fact that the patient had repeated bouts of fever, sometimes reaching 103° F., and that serous fluid could be aspirated again and again from the joint. Repeated bacteriologic studies of the joint fluid, and various agglutination tests on the blood failed to give evidence of infection. However, in the ensuing 5 weeks there was progressive destruction of the medial condyle, and the assumption of an infectious lesion became less tenable. Biopsy revealed Ewing's sarcoma of the lower end of the femur, and the limb was disarticulated. The patient died 5 months after admission, and at autopsy widespread metastases were found.

The only fairly consistent roentgenographic finding is evidence of lysis of bone, by itself a rather nondescript feature. Thus in some cases the presenting lesion may appear merely as a small zone of mottled rarefaction reflecting destruction of the spongiosa and, to a lesser degree, of the overlying cortex, associated with what is as yet only a trace of periosteal new bone apposition in reaction to the neoplastic tissue which has penetrated beyond the cortex. This picture (which may also include some areas of condensation) is very likely to suggest an inflammatory lesion (pyogenic or tuberculous osteomyelitis) rather than a tumor, but within a month or so the roentgenogram presents evidence of rapid extension of the pathologic area within and beyond the bone, now strongly supporting a diagnosis of malignant neoplasm. Although the roentgenogram still shows only a relatively small area of bone destruction, this cannot be taken as indicating the actual extent of involvement of the bone, the marrow spaces of which may already be riddled by neoplastic tissue (Figs. 1, 2, and 9).

When the initial roentgenograph of the presenting lesion shows rather clearly that one is dealing with a malignant tumor, one usually notes a large area of bone destruction, often with a large overlying soft-tissue mass. The affected area in the bone may show distention of its outline, but, if present, this is not pronounced. However, the affected area appears irregularly rarefied and mottled from the presence of smaller or larger foci of relative radiolucency and shows disruption of the cortical outline over a variable region. From a series of cases it can be stated that reactive deposition of new bone by the periosteum, where the neoplastic tissue is penetrating the cortex, is certainly not conspicuous. When, as is commonly the case, Ewing's sarcoma involves bones other than long bones, evidence of periosteal new bone apposition, although not uncommon, is not a striking finding (Figs. 3 and 4). It may not even be such in connection with involvement of long bones.

When the shaft of a long bone is the site of Ewing's sarcoma, one does not commonly observe the concentric onion-skin-like layers of periosteal new bone of a laminated pattern held to be so characteristic of the roentgenologic appearance of this tumor. Rather, a substantial portion of the shaft may show irregular mottled rarefaction, perhaps with complete absence of significant periosteal bone apposition (Fig. 5). In one of our cases of Ewing's sarcoma of the shaft of a humerus, there was considerable onion-skin-like periosteal new bone apposition, but this was not circumferential, being limited to the lateral surface, while on the medial side the bone was overlaid by a thick mass of neoplastic tissue which had broken out from the interior of the bone. In this case (Fig. 6), on the basis of the roentgenologic appearance, the

lesion was regarded clinically as an osteogenic sarcoma without evidence of exuberant new bone formation (that is, as a so-called "osteolytic" osteogenic sarcoma). In another case of Ewing's sarcoma of a humerus, the roentgenogram of the affected portion of the shaft showed an irregular moth-eaten appearance of the cortex without thickening, associated with more or less transverse streaks of radiopacity in the soft portion of the tumor mass overlying the outer surface of the bone. Again the lesion was interpreted clinically as an osteogenic sarcoma (Fig. 7).

Altogether, the only conclusions that can be drawn in regard to the roentgenographic appearance of the presenting lesion are that bone destruction (osteolysis) is the dominant feature of Ewing's sarcoma and that there is no typical appearance for this lesion. In general, Ewing's sarcoma is a tumor difficult to diagnose on a roentgenographic basis, often being mistaken in its early stage for an inflammatory lesion and in later stages for malignant tumors of other nature, including metastatic neoplasms. In many cases it may be quite difficult to make a differential diagnosis, on a roentgenographic basis, between Ewing's tumor and chondrosarcoma, "osteolytic" osteogenic sarcoma, malignant lymphoma, or metastatic neoplasms (including metastatic neuroblastoma). Sometimes, too, a solitary lesion of eosinophilic granuloma may be mistaken for Ewing's sarcoma. To make certain that a suspected tumor is Ewing's sarcoma, tissue examination is essential, but it cannot be emphasized too strongly that a pathologist confronted by a specimen taken from a suspected case for biopsy may easily be mistaken in his opinion on this basis also, especially if the tissue available is meager. However, in this connection, the error is more likely to be that of misidentifying other lesions (anaplastic carcinoma, metastatic neuroblastoma, malignant lymphoma) as Ewing's sarcoma than the reverse.

Roentgenographic Appearance of the "Metastatic" Bone Lesions

Whether the additional lesions found roentgenographically on admission or subsequently, represent metastases from the presenting lesion or are independent primary growths does not concern us precisely here. Roentgenographically, these additional lesions, like the presenting lesion, show evidence of lysis of bone. They appear first as rather faint, slightly mottled areas of rarefaction. As the resorption of the bone increases, the small, multiple, roundish foci of rarefaction become more distinct and may merge into larger, more clear-cut areas of radiolucency. In flat bones, such as those of the skull or the ilium, multiple, clear-cut, punched-out areas of rarefaction may appear in

consequence of lytic destruction of the spongiosa and overlying cortex (Fig. 8). Even a neoplastic fracture of a long bone from destructive resorption may become manifest. It is important to bear in mind, however, that the actual extent of involvement of the skeleton at any one time is never adequately reflected roentgenographically. This is true even in fatal cases in which a number of destructive lesions have been demonstrated roentgenographically in bones other than the one containing the presenting lesion. At autopsy, if many additional bones are opened, they, too, will be found to have been far more extensively invaded than was suspected from roentgenographic study of the skeleton shortly before death.

Morphologic Features

Gross Description

Our experience supports the idea that Ewing's sarcoma arises in the marrow spaces of the interior of the affected bone, rather than in the haversian spaces of the cortex or beneath the periosteum. Also, as has been indicated, anatomic examination of an affected bone will reveal much more extensive involvement than the roentgenographic or clinical findings would suggest. This can be effectively demonstrated in cases in which the presenting lesion is in a long bone, which is made available by amputation. Roentgenographically, in a femur from such a case, the disease seemed to affect only the medial condyle and the adjacent part of the shaft. The cortex in this region was fuzzy and had a superposed soft-tissue swelling, about 2 cm. in thickness. When this femur was stripped of its surrounding muscles and cut in the frontal plane, it showed neoplastic tissue not only in the medial condyle but also in the lateral condyle and contiguous portions of the shaft, in the major marrow cavity, and even in the marrow spaces of the spongiosa of the upper end. The neoplastic tissue in the region of the medial condyle and that which had penetrated beyond the cortex in this region was, for the most part, discolored by hemorrhage and interspersed with yellowish areas due to necrosis of both neoplasm and spongiosa. Elsewhere, for the most part, the neoplastic tissue was not modified by hemorrhage or necrosis, was whitish, and, notably in the major marrow cavity, took the form of massed, soft, glistening tumor nodules. Thus the diseased area clearly visible in the pre-amputation roentgenogram of this femur was merely the area in which the changes were most advanced and destructive (Figs. 1, 9, and 10). Neither the inguinal nor the popliteal lymph nodes were enlarged or involved by tumor. Furthermore, in spite of the extensive implication of the femur,

no tumorous involvement was discernible in the tibia, fibula, or bones of the foot, all of which were opened and examined. These negative findings are of interest because at autopsy, 3½ months after the disarticulation of the right lower limb, widespread involvement of the skeleton and visceral metastases were found.

In contrast to a small tumor mass beyond the limits of the bone proper, as demonstrated in the femoral lesion just described, one finds, relatively often, a very large tumor mass beyond the limits of the bone, as part of the presenting lesion. This was well demonstrated in the case of a young girl whose presenting lesion was in the left ilium and whose complaints were of only a few days' standing at the time of admission to the hospital, but who then showed a firm tumor mass of the size of a neonate head, fixed to the ilium and palpable in the lower quadrant and groin. Although this patient appeared to be in good general health on admission, roentgenograms showed pulmonary metastases, and she died 4 months later. So far as the presenting lesion was concerned, autopsy revealed a huge mass overlying the inner surface of the left innominate bone, which had pushed the urinary bladder and the genital organs anteriorly and the sigmoid and rectum medially. On removing the left innominate bone, it was found that the tumor had extended posteriorly through the ilium and was bulging into the gluteal muscles and penetrating the capsule of the hip joint. The extra-osseous neoplastic tissue was soft, friable, extensively hemorrhagic, spongy and cystic on the whole, and in many places almost diffluent. The iliac bone was riddled by neoplastic tissue which was cystic in many places and there were many defects in its cortex, both on the inner and outer aspects, from which, as noted, the tumor had spread beyond the limits of the ilium proper into the surrounding soft tissues (Figs. 3 and 11).

As previously mentioned, 4 of the 17 cases upon which this report is based were autopsied. Two of the autopsies were performed by us. The other two were performed at Montefiore Hospital, New York City, and we are indebted to Dr. Samuel H. Rosen of the Laboratory of Pathology of that hospital for the opportunity of studying the protocols and slides. These autopsies were carried out with full awareness of the general lack of thorough autopsy studies in cases of Ewing's sarcoma.

At the time of death, 2 of these patients were 16 years of age; the other 2 patients were 18 and 19 years old, respectively. Three were females. The youthfulness of these patients makes it improbable that the skeletal lesions represented metastases from a carcinoma, although the possibility cannot be excluded. If we were dealing in these cases with metastases of carcinoma from an unrecognized primary growth,

whatever its site and however anaplastic the metastases might be, it is highly improbable that these lesions would consistently present the cytologic pattern peculiar to well preserved Ewing's sarcoma, to wit: cells of rather uniform size, with ill defined borders, little cytoplasm, and fairly large and rather uniform roundish or oval nuclei showing scattered chromatin.

Also, the findings, at least in the 2 cases (one male and one female) which we personally autopsied, clearly ruled out the presence of a hidden carcinoma. The breasts and testes were carefully searched for neoplastic tissue and none was found. In both cases, the lungs were given particular attention. In one no grossly visible tumor nodules were found anywhere in the pulmonary parenchyma. The bronchi, which were opened to the very small branches, as well as the hilar lymph nodes showed no gross evidence of involvement, reducing the likelihood that we had overlooked a primary bronchial carcinoma. In the other case, although all lobes of the lungs were riddled with hundreds of soft, richly cellular, largely hemorrhagic and liquefied tumor nodules of various sizes, they showed no single massive tumor, and it was plain from the gross appearances that the lesions were metastatic. In the course of the autopsies, full consideration was given to the fact that the gastrointestinal tract, and especially the stomach, can be the site of unsuspected carcinoma, but neither the gastrointestinal tract nor the lymphoid tissue regional to it showed tumorous involvement. Every precaution was taken to exclude the possibility that one was dealing not with a tumor primary in the skeleton but merely with metastases to the skeleton from a carcinoma which, in its primary site, was overlooked because it was inconspicuous or, although observed, was misinterpreted as a metastasis.

In a case thought to represent Ewing's sarcoma, the problem posed by neuroblastoma (primary in the adrenal medulla or in sympathetic nervous tissue elsewhere) is an even greater challenge than that raised by carcinoma. Sympathicoblastoma must always be ruled out in such a case, since this tumor not only has a strong tendency to metastasize widely to the skeleton but may bear a confusing cytologic resemblance to Ewing's sarcoma. This point was rightly stressed by Willis,[8] although he has often been misinterpreted as rejecting entirely the entity of Ewing's sarcoma and holding that all such cases represent merely metastases, particularly from neuroblastoma. Be that as it may, the adrenals in all 4 of our autopsied cases failed to show, on detailed gross examination, evidence of tumorous involvement or of any other abnormality. In the 2 cases which we autopsied personally, an extended search of the areas around the adrenals and of the sym-

pathetic chains along the vertebral column failed to show evidence of an extra-adrenal sympathicoblastoma.

As to the viscera, we have already pointed out that the lungs grossly may be found free of neoplastic tissue or, on the contrary, riddled with metastatic nodules. Under the latter conditions, we also have found the parietal pleura studded with tumor masses, some of which were large and fungating. In 2 cases, the liver presented numerous metastases, mainly in the form of nodules a few millimeters to somewhat more than a centimeter in diameter. In one case or another, metastases were noted in one or more of the following organs: heart, spleen, kidneys, pancreas, and thyroid. Finally, it should be noted that the lymph nodes, by and large, tended to be free of neoplastic tissue, although in one case some of the paravertebral and pelvic lymph nodes showed, microscopically, some nests of tumor cells in the peripheral sinuses as extensions of the neoplasm from the underlying vertebrae. The striking lack of involvement of the lymph nodes is additional evidence against the possibility that an occult primary carcinoma or neuroblastoma was present.

As already indicated, one can expect to find at autopsy that much of the skeleton, in addition to the bone with the presenting lesion, is affected, and much more extensively than one would have suspected from the ante-mortem roentgenographs. The question which cannot be answered definitely is whether the wide dissemination through the bones represents metastatic spread of the neoplasm or its autochthonous appearance in multiple sites.

At any rate, the calvarium is likely to show the neoplasm permeating the diploic spaces, and, in addition, areas in which neoplastic tissue has eroded or completely destroyed the tables. In the latter case, the calvarium will show actual defects, frequently several centimeters in diameter, filled with cellular, gray white or even greenish yellow neoplastic tissue which may elevate or even penetrate the regional calvarial coverings. The marrow spaces of the ribs and sternum, too, are likely to be filled with neoplasm, and thinning and erosion of the cortex may be associated with focal masses of neoplastic tissue beneath the periosteum. In both cases which we autopsied, large sections of the vertebral column were removed, and here too we found the marrow spaces of the bodies, arches, and spinous processes extensively infiltrated. In one case in particular, practically every dorsal and lumbar vertebral body showed areas in which the neoplasm in the marrow spaces and the supporting spongy bone appeared yellowish in consequence of necrosis (Fig. 12). Where there was no necrosis, the neoplastic tissue was grayish, soft, and obviously cellular. In many places the neoplastic

tissue was extending through the bodies and beneath the anterior vertebral ligament. From the third dorsal segment to the first lumbar segment, the new growth had also extended beneath the dura, narrowed the spinal space, and cuffed and compressed a large section of the spinal cord, with resultant degeneration of the latter.

Microscopic Description

Although Ewing's sarcoma does have a characteristic cytologic pattern (as Melnick [17] also maintained), secondary changes may obscure it or make it difficult to demonstrate in an individual specimen taken for biopsy, even if it has been obtained by surgical incision. Thus, a specimen may show large fields in which the appearance of the individual tumor cells has been altered by degeneration and necrosis, areas in which the neoplastic tissue as a whole has been modified by hemorrhage and reparative reaction to it, and even areas in which reactive inflammatory changes dominate the picture. It is because such secondary changes are not relegated to the background that the reputation of Ewing's sarcoma for variability and inconstancy of its cytologic pattern in biopsy specimens from case to case has developed and persists.

However, secondary changes in the neoplastic tissue do not present the only difficulty with which one is confronted in attempting to make a diagnosis of Ewing's tumor on the basis of a biopsy specimen. The diagnosis "Ewing's sarcoma" often has become a mere refuge when one is confronted by a puzzling malignant tumor in a bone, and is likely to be applied rather loosely and by default of a better opinion unless one's anatomic conception of Ewing's sarcoma is definite. This has been discovered by others, too, when they have re-evaluated their cases. It was brought home to us by restudy of all the material (27 cases) listed in our files under the heading of Ewing's sarcoma during the past 20 years. Among the cases so listed, there were some in which the material was so poor in well preserved tumor cells that we would now hesitate to make the diagnosis of Ewing's sarcoma on that basis alone. Although some of the lesions may have been Ewing's sarcomas, we must have been largely guided by the clinical, and especially the roentgenographic, findings in arriving at that diagnosis. There were also a number of cases in which restudy showed that our original diagnosis of Ewing's sarcoma had been incorrect, and, specifically, that we had erroneously included under this diagnosis some cases in which the bone lesion under consideration was actually a myeloma, a lymphocytic lymphoma, an anaplastic metastatic carcinoma, or a metastatic neuroblastoma. After excluding all cases in which the available tissue was defective in quality or inadequate in amount and those in which the

original diagnosis now seems clearly to have been erroneous, there remained the 17 cases upon which the present report is based.

The characteristic cytology of Ewing's sarcoma, irrespective of the source of the neoplastic tissue, is manifested through the presence of smaller or larger fields of tumor cells which lack clearly delimited cell boundaries, the nuclei being crowded together and of fairly uniform appearance. These nuclei are round or ovoid, are about twice as great in diameter (or, in the case of the ovoid ones, perhaps three times as great in the longer axis) as the nucleus of a lymphocyte, and have finely divided or powdery chromatin and often one or more nucleoli. As a rule, the individual nuclei appear enmeshed in, and slightly separated by, a loose, more or less vacuolated cytoplasmic fabric. In some fields, however, they may be found crowded together (perhaps to such an extent that many of them are even pressed into an oval shape), and in such fields there is but little cytoplasm between them. It should also be noted that in the fields presenting the general cytologic picture just described, vascularity is usually not a prominent feature (Figs. 13 and 14).

Cellular areas showing the characteristic cytology described above have to be searched for in the specimen taken for biopsy from the presenting bone lesion in an individual case, since the neoplastic tissue may have undergone abundant secondary changes, such as degeneration and necrosis. Degeneration is indicated in the nuclei by pyknosis and reduced size, and such nuclei are likely to be surrounded by a narrow zone of cytoplasm with a delimiting cell border. It is such cells that approximate most closely the picture given by Ewing for the type cell, to wit: a small polyhedral cell with pale cytoplasm, a small hyperchromatic nucleus, and a well defined cell border. Intermingled with the fields in which the tumor cells are undergoing degeneration there are usually areas in which the cells have undergone necrosis. Degenerating, but particularly necrotic, neoplastic tissue may, in some places, be heavily infiltrated by polymorphonuclear leukocytes (Fig. 15). Hence, if only a limited fragment of tissue is examined and sufficient care is not taken, this picture may be misinterpreted as representing an infectious process rather than a tumor. In connection with the presence of polymorphonuclear leukocytes in degenerating and necrotizing neoplastic tissue, we have found no evidence in favor of the concept once suggested that Ewing's sarcoma usually arises in marrow previously altered by fibrosing or sclerosing osteomyelitis.

Free hemorrhage into fields of neoplastic tissue, especially if it is extensive, comes to be associated with the ingrowth of many blood

vessels into those fields. If the neoplastic tissue in these areas is not necrotic, one will note that many of these vessels are collared by tumor cells. However, the vessel spaces are not lined by tumor cells, and between the latter and the lining cells there is tissue representing the wall of the vascular space. Thus, while it is true that in such fields one does see tumor cells about capillary spaces or around larger vascular spaces in a so-called "perithelial" arrangement, one does not see this orientation of tumor cells to any pronounced extent except in connection with hemorrhage. It is on this account that no distinctive cytologic significance attaches to such findings. Similar perivascular orientation of tumor cells is observed in connection with sarcomas of other kinds in which focal areas have undergone extensive hemorrhage (Fig. 16).

We turn now to the question of the presence of rosette (or pseudorosette) formations. In connection with an occasional Ewing's sarcoma, authors have reported and illustrated formations in which cells are arranged circularly (although not around a vessel) in so-called rosette formation. However, in such illustrations it can be seen that the centers of these formations represent degenerated cells with shadows which are still perceptible, rather than fibrillar or granular cores as in neuroblastoma. We think that the "pseudorosettes" illustrated by Gharpure [18] in his case of Ewing's sarcoma clearly show that the core about which the viable tumor cells are circularly disposed represents a mass of necrotic tumor cells, the outlines of which are still plainly visible. Also, the "rosettes" illustrated in the case presented by Foote and Anderson [19] seem likewise to appear in tissue fields where cells are undergoing necrosis. In our own material, we also have occasionally encountered a tumor field in which viable tumor cells were disposed about cores of degenerating cells. In one case this was a rather prominent feature, but even then the formations were not clearly suggestive of the rosettes of neuroblastoma (Figs. 17 and 18).

Finally, we come to the question of reticulum fibrils in Ewing's sarcoma. It appears that these are not a consistent nor a prominent feature of the histologic picture. Indeed, there is considerable variability in regard to these fibrils, from lesion to lesion and even from part to part of the same tumor section. Some of the lesions, in part or throughout, have at most only a few stray argyrophil fibrils in an entire low-power field. Other lesions show more numerous fibrils, but even in them the fibrils are irregularly distributed and are seen only between smaller and larger groups of tumor cells. In no tumor did we regularly see large fields of tissue showing a lattice or meshwork of reticulum fibrils outlining not merely cell groups, but the individual

tumor cells. In view of this variability, it is clear that there is no characteristic histologic pattern for Ewing's tumor in so far as these fibrils are concerned.

The Problem of Primary Reticulum Cell Sarcoma of Bone in Relation to Ewing's Sarcoma

In 1939, in an article entitled "Primary Reticulum Cell Sarcoma of Bone," Parker and Jackson [14] called attention, on the basis of 17 cases, to a malignant bone tumor which they held to be distinctive and, in particular, to be different from Ewing's sarcoma, with which it is most often confused. They believed this tumor to be derived from the reticulum cells of the marrow of the affected bone, and indicated that the cell type of this tumor was identical with that of reticulum cell sarcoma of lymph nodes and other tissues, and that diagnosis of the condition must rest upon tissue examination. As to the histology of the tumor, they stated that the cell nucleus, which is from one and one-half to two times larger than in a lymphocyte, varies in shape from round to oval; frequently, it may be indented or lobulated. The chromatin may be finely divided and scattered, or, on the other hand, it may be coarser and nucleoli may be present. There may be considerable cytoplasm about the nucleus. Evidence of ameboid activity, as indicated by the oval or elongated shape of the cell and its nucleus, is frequently present and is a characteristic feature. Binucleate forms occur, but true tumor giant cells do not. Mitotic figures are often present in large number. When the neoplastic tissue is stained in order to bring out the reticulum, the latter is found to run in delicate threads and strands around groups of tumor cells and also between individual cells.

Thus, on cytologic grounds, there seem to be some tangible, although not striking, differences between the tumor described by Parker and Jackson [14] and Ewing's sarcoma. Stout,[13] however, found himself unable to distinguish between the two even on a cytologic basis, holding that they are simply variants of the same tumor. On the other hand, it is evident from the revised (1939) classification by the Registry of Bone Sarcoma of the American College of Surgeons that Ewing [11] accepted the concept of primary localized reticulum cell sarcoma of bone as a tumor entity, and equally evident that he held it to be distinct from the Ewing tumor. The article by Edwards [20] supplied further support for the concept of primary localized reticulum cell sarcoma of bone and gave details of a case which was followed through to autopsy. The article by Gall and Mallory,[21] although devoted to the problem of malignant lymphoma in general, likewise sustained this

thesis and pointed out that sometimes a malignant lymphoma arises as a solitary lesion in the marrow of a single area of bone, and tends to remain localized there for a long time before spreading even to the adjacent lymph nodes. In particular, Gall and Mallory indicated that among the relatively infrequent instances in which a malignant lymphoma takes this clinical form, cases of clasmatocytic lymphoma (one of their two varieties of so-called reticulum cell sarcoma) are prominent. In their Table IV (page 404), they list 6 cases of clasmatocytic lymphoma which appeared to be initially localized to a bone.

Clinically, a primary reticulum cell sarcoma of bone presents itself as a painful, destructive lesion, often extensive, but localized to the area of bone in question, while the general health of the patient is good. When it is a long bone that is affected (as is most often the case), the end and much of the shaft are usually involved, and a neoplastic fracture may have resulted. The roentgenographic picture is not distinctive, showing only that one is dealing essentially with an osteolytic lesion, which may have broken through the outer bounds of the cortex and extended into the surrounding soft parts. In this respect, the roentgenographic picture is interchangeable with that of Ewing's sarcoma. As to treatment, Parker and Jackson [14] stressed the practical value of early diagnosis (by biopsy) followed by immediate amputation or ablation if the lesion is in a site permitting this, and otherwise by wide local excision if possible. They further advocated that this should be followed by local radiation therapy, but pointed out that such therapy by itself is inadequate. Of the 17 patients whose cases are discussed by them, 7 who received appropriate treatment have been apparently free from tumor for 10 years or more. In several of the other cases death supervened, but in none of these was an autopsy done. However, there were indications that late in the course of the disease the tumor may extend to the regional lymph nodes and even spread distantly by way of the blood stream.

It is a knotty problem to decide whether, and if so to what degree, one should differentiate between primary reticulum cell sarcoma of bone and Ewing's sarcoma. Certainly, in view of its reliable sponsorship, the concept of reticulum cell sarcoma of bone should not be lightly pushed aside. If further clinical experience supports the current observation that primary reticulum cell sarcoma of bone has a definitely more favorable prognosis (if correctly and promptly treated) than Ewing's sarcoma, there will be a good practical reason, also, for preserving the distinction. If, in a given case, the cellular structure favors a diagnosis of reticulum cell sarcoma of bone and there is as yet no clinical evidence of distant spread of the tumor, one would be all

the more justified (when the lesion is in a suitable site) in urging prompt amputation or ablation of the affected part.

The Problem of Neuroblastoma with Skeletal Metastases in Relation to Ewing's Sarcoma

The fact that a sympathetic neuroblastoma (sympathicoblastoma) commonly metastasizes to bones has been known for a long time. Hutchison,[22] and Tileston and Wolbach [23] have pointed out that one can anticipate finding, at least at autopsy, a malignant adrenal tumor as the primary lesion in infants and children clinically presenting tumorous involvement of cranial bones, associated with proptosis from tumorous involvement of the orbital region and tumorous enlargement of the preauricular and other regional lymph nodes. From the cases reported by these authors, and from those which they collected from the literature (cases now assignable to adrenal neuroblastoma), it was evident that metastases to bones other than those of the skull are also often found, and that metastases to the liver, kidneys, and lymph nodes in general were among the other common findings.

Further progress in the understanding of sympathetic neuroblastoma has revealed that, although the adrenal medulla is the most common site of origin for these tumors, it is by no means the only one. Cases have been reported in which they arose from some part of the sympathetic nervous tissue elsewhere in the body, notably from the sympathetic chains, but sometimes even from the sympathetic tissue of organs. While infants and young children are the most common victims, occasional instances have been reported in which sympathetic neuroblastomas developed in adults. Also, it has become clear that in so far as the skeleton is concerned, the clinically presenting, destructive bone lesion (if there is one) may be in a long bone or some bone other than the skull.

In respect to cytology, Tileston and Wolbach [23] stressed the diagnostic significance, for the condition in question, of the finding (in various numbers) of tumor cells arranged in rosettes. It was Wright [24] who pointed out that these tumors take their origin from the pluripotential cells of the sympathetic nervous system, and that the rosettes are ball-like aggregations of tumor cells enclosing a small central meshwork of filamentous neurofibrils, some of which can be seen to constitute processes of the cells making up the periphery of the rosette. In addition, he pointed out that, aside from rosettes, one may be able to find, as also peculiar to neuroblastoma, masses of tumor cells interspersed with and penetrated by fibrils running parallel in bundles. But the demonstration of neurofibrils, either in parallel bundles or as a meshwork in the center of the rosettes, may be difficult. Specifically,

in a given case, few fibrils may have been laid down, or, by degeneration or post-mortem change, such fibrils as were laid down may have become transformed into hyaline or granular material and be difficult to demonstrate on this account. This is especially true of the fibrils of the rosettes. Under such circumstances, whatever rosettes are present appear as formations in which several rows of cells surround a finely granular, eosin-staining mass without a central lumen.

In a particular case, rosettes may be fairly numerous in both the primary growth and the metastases, conspicuous in the primary growth and sparse in the metastases, or difficult to find in either. As to the type cell of the tumor, there are differences from lesion to lesion, depending on the predominating level of maturation. In the most primitive type, the dominating cell maintains the lymphocytoid character of the parent stem cell. This cell is thus a small round cell (strongly resembling a small lymphocyte) with a dense hyperchromatic nucleus practically filling the entire cell so that there is little cytoplasm. Some of the cells, although maintaining this general character, may be oval, while others, especially at the periphery of the rosettes, may be piriform. In more differentiated sympathetic neuroblastomas, the cells, although mainly round, are distinctly larger than those just described and may have vesicular nuclei and a clear ring of cytoplasm about the nucleus, and even some cytoplasmic processes. In still further matured neuroblastomas, some tumor fields may even show sympathetic ganglion cells.

Against this background, we are in a position to understand Willis' point of view on neuroblastoma in relation to Ewing's sarcoma. Prior to the publication of the first relevant article by Colville and Willis [7] in 1933, it seems not to have been adequately stressed that care must be taken to exclude the possibility that one may be dealing with a sympathetic neuroblastoma metastatic to the skeleton in cases supposedly representing Ewing's sarcoma of bone. In that article a case is detailed (as is another one in 1940 [8]) in which there was a presenting tumor in a femur which had the usually accepted clinical and roentgenographic characteristics of Ewing's sarcoma. In these cases the clinical course, and in particular the susceptibility of the tumor to radiation therapy, seemed to support this diagnosis. It should be pointed out that in neither case were rosettes found in the material taken for biopsy from the femoral lesions. However, in both cases it was revealed at autopsy that the femoral tumor was a metastasis from a neuroblastoma, primary in an adrenal in one instance and in the left lumbar sympathetic chain in the other. In both cases rosettes were found only in the primary growth.

On the basis of these experiences, Willis [8] expressed great wariness

about a diagnosis of Ewing's sarcoma made on clinical (including roentgenographic) grounds alone. He cast doubt also upon the reliability of biopsy in this connection, and analyzed, largely to reject them, the findings in the relatively few cases published prior to 1940, which had been interpreted as Ewing's sarcoma proved by autopsy. His paper of 1940 bears careful reading for its evaluation of the reported autopsied cases of Ewing's sarcoma, even if it does appear that in some instances he has been over-critical in the standards he set.

There can be no doubt that Willis [8] was correct in holding that a presenting bone lesion which is in fact a metastasis of neuroblastoma may not be recognized as such on the basis of biopsy, and the following case from our own material illustrates this. The patient was a boy, 3 years of age, who was admitted because of pain in the left hip region and limping of 3 months' duration. A roentgenogram revealed a rarefying lesion in the neck of the left femur, resorptive destruction of the cortex in this area, and some periosteal new bone deposition on the cortex of the adjacent portion of the femoral shaft (Fig. 19). On the assumption that the lesion was a low-grade osteomyelitis, it was curetted, but this assumption could scarcely have been made if the child had been studied thoroughly before surgical intervention, for, on admission the child already presented a dilated left pupil, and evidences of general lymphadenopathy, especially prominent in the left cervical region. The tissue sections from the material curetted from the neck of the femur showed a malignant tumor. The tumor cells were supported in a connective tissue stroma which was loose in some places, rather collagenous in others, and tended to demarcate smaller or larger groups of the cells. The predominating type of cell was rather large and round, lacking a clear-cut cytoplasmic outline and having a large, pale, stippled nucleus. Although some smaller cells with dark hyperchromatic nuclei were present, a number of tumor giant cells, some of which had two or more nuclei, were also seen. The cytologic picture (Fig. 20) was not that which we associate with Ewing's sarcoma, nor, on the other hand, was it even vaguely suggestive of neuroblastoma. However, that the femoral lesion was in fact a metastatic neuroblastoma could be safely deduced from histologic examination of several enlarged lymph nodes from the left cervical region, which showed unmistakably the rosettes and other cytologic features of sympathetic neuroblastoma (Fig. 21). Unfortunately, we could not determine the site of origin for the neuroblastoma in this case, since there was no autopsy. The child died at home about 1 year after the onset of the complaints.

This case of sympathicoblastoma was peculiarly difficult to diagnose,

and makes us sympathetic to Willis' [8] contention that only carefully executed autopsies can prove or exclude neuroblastoma or completely justify a diagnosis of Ewing's sarcoma. However, that a large proportion of cases of sympathetic neuroblastoma have certain distinctive clinical and roentgenographic features which are useful in differentiating them from Ewing's sarcoma can be gathered from the mass of material on which Wyatt and Farber [25] have reported. As to our own 13 cases in which the diagnosis of Ewing's sarcoma was based on biopsy findings, it can be said that, in view of the uniformity of the cell type in these cases and its consistent resemblance to the cell type observed in the 4 autopsied cases, we feel reasonable confidence in assuming that we could not have been dealing in all 13 cases with metastases from neuroblastomas. This assumption seems all the more justified if one bears in mind that: None of the many tissue sections cut in these 13 cases showed the rosettes classic for neuroblastoma; if these 13 lesions represented metastases from neuroblastomas, the primary tumor in all these cases would have had to be silent and the cells in all the lesions would have had to be matured to, and only to, the sympathoblast level; all but one of these 13 patients was over 8 years of age, whereas the great majority of cases of neuroblastoma are seen in children under this age.

The Problem of Carcinoma and Other Malignant Tumors with Skeletal Metastases in Relation to Ewing's Sarcoma

The problem of the differential diagnosis of Ewing's sarcoma does not end with sympathicoblastoma, but may be raised also by metastatic carcinoma. That a solitary destructive bone lesion which is proved by biopsy to represent a metastasis may be the first clinical indication that the patient is suffering from carcinoma hardly needs to be stated. It is also common to find that, while the primary lesion is silent, the histologic picture of the neoplastic tissue in the biopsy specimen gives the clue to the site of the primary growth. Often, on the other hand, there is not sufficient cytologic differentiation to suggest the site of the primary lesion. Diagnostic difficulties arise in those cases in which the primary growth is silent and in which the neoplastic tissue in the metastatic focus is so undifferentiated as to present a more or less uniform pattern of round cells. Although this problem does sometimes arise in connection with biopsy diagnosis or even in connection with the evaluation of the autopsy findings in a suspected case of Ewing's sarcoma, it does not constitute a frequent or serious difficulty in the hands of an experienced pathologist. Still, Hirsch and Ryerson [26] pointed out that bronchial carcinomas (particularly small ones with

undifferentiated cells) may metastasize widely to the bones before being recognizable in the lung and thus raise problems of differential diagnosis from Ewing's sarcoma, a point of view also stressed by Sternberg.[27] Sternberg also cited a case in which a skeletal metastasis was regarded as Ewing's sarcoma, although he himself held that involvement of bone was secondary to an undifferentiated small-celled carcinoma of the breast.

Finally, it may not be amiss to point out that occasionally, in the course of evaluation of a biopsy specimen, one may have to make a differential diagnosis between Ewing's sarcoma on the one hand and Hodgkin's disease and lymphocytic lymphoma on the other. However, the latter conditions are so rarely primary in bones that one is not often confronted by this problem as a practical difficulty, and when they are not primary there the general clinical picture, in which involvement of lymph nodes occupies the foreground, helps to clarify the problem.

Prognosis and Treatment

With the exception of one patient, who was admitted to the hospital only 5 months ago and whose course is already downhill,* all of the patients in our series of 17 cases of Ewing's sarcoma have died. Some died within 6 months to a year, and all but 3 were dead within 3 years of the onset of the local clinical complaints. Of the 3 who survived longer, 2 lived for 3½ years and one for 5½ years after the onset of complaints. If calculated from the time of admission to the hospital, the period of survival is, of course, somewhat shorter in all cases. This doleful prognosis is also evident from the Memorial Hospital statistics recently cited by Coley,[28] who stated that of 71 cases there were none in which survival was beyond 5 years.

All of our patients received radiation therapy to the presenting bone lesion. However, it should be noted that these cases accumulated over the past 20 years, during which a good deal of progress has been made in radiation procedure, so that the results cannot be judged on a uniform basis. Also, in many of our patients, the presenting lesion was in a bone of the trunk, where the advantages of a large radiation dose are frequently more than counterbalanced by the danger of damage to internal organs. In addition, in those cases which ran a rapidly down-

* This patient died in August, 1946 (13 months after admission to the hospital) and autopsy amply confirmed the diagnosis of Ewing's sarcoma. Examination included a search for all possible primary sources of neuroblastoma. It further included the calvarium, practically all of the vertebral column (along with the sacrum), both innominate bones, the upper ends of both femora, part of a tibia, and many ribs. The marrow spaces of these bones were found extensively invaded by neoplastic tissue, which, where viable, showed the cellular characteristics of Ewing's sarcoma.

hill clinical course, it was almost certain that widespread metastases were already present at the time when radiation therapy was instituted and necessarily contributed to its ineffectiveness.

Inferences as to the influence of radiation therapy alone in the treatment of Ewing's sarcoma can best be drawn from those cases in which the disease is apparently still limited to a long bone. It is in these cases that cross-fire radiation of the whole affected bone from every possible angle is feasible, with a total tumor dose of as much as 4500 r. in one course of treatment, as advocated by Swenson.[5] Yet, in the 2 cases in our series which were adequately treated along these lines (one lesion in a fibula and the other in a humerus), and in which radiation therapy had a remarkable palliative local effect, the patients nevertheless succumbed to metastases 3½ and 5½ years, respectively, after the onset of the clinical complaints. Thus it would seem that radiation alone cannot be counted upon to produce a cure even in favorable cases. In such cases, radiation followed by surgery (amputation or disarticulation of the limb) may give better results, but our material includes no cases in which this combination has been tried. On the whole, we believe that even under the most favorable circumstances the ultimate prognosis in cases of Ewing's sarcoma is, as yet, very bad.

Summary and Conclusions

This study (based on 17 cases, 4 of which were autopsied) supports the existence, among the primary malignant tumors appearing in bones, of a tumor entity to which, because of Ewing's pioneer effort to single it out, the name of Ewing's sarcoma should be applied. Beyond the fact that it is a specific malignant tumor primary in bones, and that its cells show no osteogenic potentialities, there is still much to be learned in respect to its histogenesis. Study of the cytologic patterns in our material yields no support for Ewing's contention that the neoplastic cells are derived from capillary or vascular (or perivascular) endothelium. It is true that tumor areas which have become heavily invaded by blood vessels, especially in the wake of hemorrhage, often show tumor cells about capillary spaces or around larger vascular spaces in a so-called "perithelial arrangement," but perivascular orientation of the tumor cells is not a characteristic cytologic feature of this neoplasm. Also, when, in an occasional lesion, one finds formations in which cells show a ring-like arrangement (though not around a vessel), these formations can be seen to have resulted from degeneration of centrally located cells, the shadows of which are still perceptible. Such formations really have nothing in common with the rosette or pseudo-rosette formations of neuroblastoma.

We incline toward Oberling's idea that the tumor cells of Ewing's sarcoma are derived from the supporting framework (the reticular tissue) of the bone marrow, a framework which can be regarded as a mesenchymal or primitive form of connective tissue. Ewing described the type cell of the lesion as a small polyhedral cell with pale cytoplasm, a small, hyperchromatic nucleus, and a well defined cell border. However, as revealed in viable, well fixed and well stained neoplastic tissue, the type cell is actually found to have an ill defined cell border, little cytoplasm, and a fairly large, round or oval nucleus showing scattered chromatin. Nevertheless, to make a diagnosis of Ewing's sarcoma from a biopsy specimen, even if the latter is obtained by surgical incision, is sometimes difficult, because of secondary changes which the neoplastic tissue has undergone. Cell areas showing the characteristic structure are sometimes to be found only after many sections have been made and examined.

A diagnosis of Ewing's sarcoma on the basis of biopsy should not be made without giving consideration to the possibility that one may be dealing with a sympathetic neuroblastoma or anaplastic carcinoma metastatic to the affected bone. Such alternative possibilities as primary reticulum cell sarcoma of bone, Hodgkin's disease, malignant lymphoma, and even myeloma must be eliminated. If, in a patient suspected of having Ewing's sarcoma, enlarged lymph nodes are palpable (regionally to the affected bone, or elsewhere), these too should be examined anatomically, in consideration of alternative possibilities, since lymph nodes are not commonly involved in Ewing's sarcoma, at least in an early stage.

On the clinical side, in our cases of Ewing's sarcoma we found that the great majority of the patients were in the second decade of life. The clinical histories did not show trauma to be an instigating factor. In the majority of our cases, the presenting lesion was in a bone of the trunk. We found no evidence favoring the idea that the presenting bone lesion shows a characteristic, if not typical, roentgenographic picture of high diagnostic value.

Ewing's sarcoma has a most doleful prognosis, only one of the 17 patients in our series still being alive, and this one has been under our observation for only 5 months.* Fever, secondary anemia, and an increased sedimentation rate of the blood in a patient with Ewing's sarcoma are evidences that the course will be a fulminating one, ending in death within a few months. Radiation therapy alone, while often having a remarkable palliative local effect for some time, offers as yet but little hope so far as the ultimate issue is concerned. The combi-

* See footnote on page 64.

nation of radiation therapy with surgery in favorable cases would seem to be more promising, but has not yet received sufficient trial to warrant a statement about its effects.

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DESCRIPTION OF PLATES

PLATE 8

FIG. 1. Roentgenograph showing rarefaction of a medial femoral condyle and of the cortex of the shaft just above the condyle. There is also an indistinct, small, soft tissue mass overlying the cortex above the condyle, the faint radiopacity representing periosteal new bone apposition. Although the picture suggests that the lesion might be a malignant tumor, there is nothing in it to justify the specific conclusion that it represents Ewing's sarcoma. The limb was disarticulated a week later, and Figure 9, showing the femur in coronal section, reveals how much greater was the actual involvement than was apparent roentgenographically. The patient, a male of 19 years, died 5 months after admission to the hospital, and an autopsy was done.

FIG. 2. Roentgenograph showing irregular rarefaction of the neck of a femur. The cortex is relatively unmodified and there appears to be no soft tissue mass overlying it. It would hardly be suspected from this picture that the lesion was a neoplasm. On a clinical basis, it was thought to be tuberculosis. Surgical intervention undertaken on this premise revealed Ewing's sarcoma. The patient, a boy of 14 years, died at another hospital 4 months after admission to our hospital, and no autopsy was done.

FIG. 3. Roentgenograph showing rarefaction and disruption of the lateral cortical outline of an iliac bone—a picture pointing clearly to the presence of a malignant tumor, although not necessarily Ewing's sarcoma. This was the picture of the presenting bone lesion on admission, and, although local clinical complaints were of only several days' standing, a tumor mass of the size of a neonate head could already be felt attached to the iliac fossa and the lateral aspect of the ilium. Pulmonary metastases were already present. The patient, a girl of 16 years, died 4 months after admission to the hospital, and an autopsy was done. Figure 11 shows the removed affected innominate bone in longitudinal section through the region of the acetabulum.

FIG. 4. Roentgenograph showing an extensive, destructive, rarefying lesion, involving the body and ascending ramus of a pubic bone and definitely suggesting a malignant neoplasm. This was the picture of the presenting bone lesion on admission, and the clinical complaints referable to the ipsilateral hip joint were already of 8 months' duration. At this time, lesions were evident in some other bones also and in the lungs. The patient, a girl of 17 years, died at another hospital about 8 months after admission to our hospital, and no autopsy was done.

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Ewing's Sarcoma of Bone

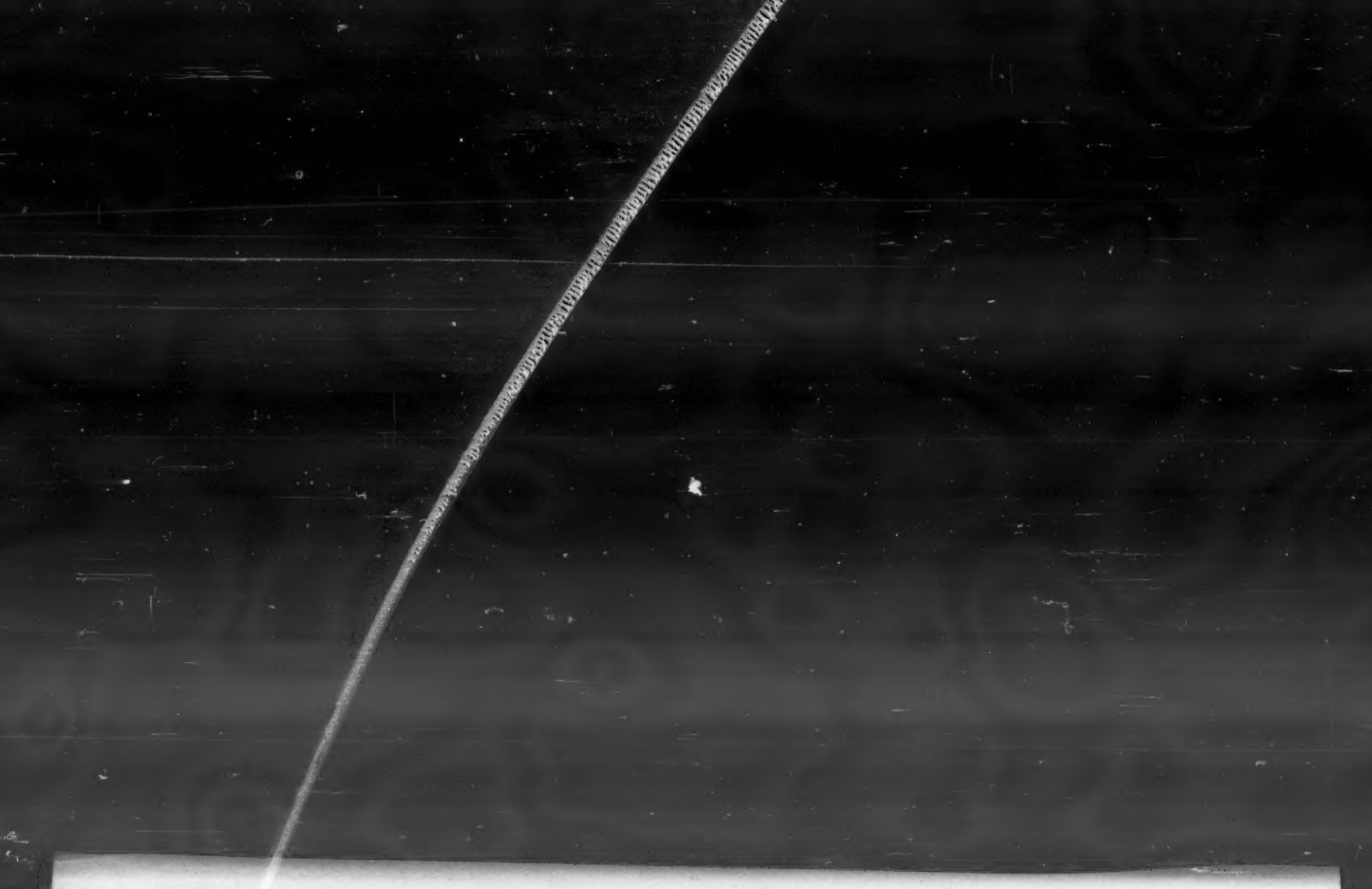
PLATE 9

FIG. 5. Roentgenogram showing extensive mottled rarefaction of the upper half of a tibia without any significant deposition of new bone by the periosteum. Although the picture of this presenting lesion could suggest a malignant tumor, there is nothing in it to justify, by itself, the conclusion that the lesion represents Ewing's sarcoma. Clinically it was suspected that it had an inflammatory basis. However, it was a Ewing's sarcoma and the patient, a girl of 18 years, died 11 months after admission to the hospital. An autopsy was done at Montefiore Hospital.

FIG. 6. Roentgenograph showing extensive destructive rarefaction of the shaft of a humerus, associated with onion-skin-like periosteal new bone deposition in the lateral side and a thick mass of neoplastic tissue overlying the bone on its medial side. This mass, in its upper region, presented some transverse radiopacities. The picture is clearly that of a malignant tumor, but if one had to be guided by the roentgenograph alone one would say that the lesion was an "osteolytic" osteogenic sarcoma rather than a Ewing's sarcoma. The patient was a girl, 8 years old, who died at home 7 months after admission to the hospital, and no autopsy was obtained.

FIG. 7. Roentgenograph of the upper portion of the shaft of a humerus. The cortex is not thickened but presents an irregular, moth-eaten appearance, while the soft tissue tumor mass overlying it shows more or less transverse streaks of radiopacity. This picture, too, suggests an osteogenic rather than a Ewing's sarcoma. The patient was a male, 24 years of age, who died at home 5½ years after admission to the hospital, and no autopsy was obtained. (See also Fig. 8.)

FIG. 8. Punched-out rarefactions in the calvarium of the patient referred to in the legend for Figure 7, representing lytic destruction of the bone by neoplastic tissue. This was the appearance of the calvarium 6 months before the patient died. It is a nondescript appearance which could have been created also by plasmatocytic myeloma or metastatic carcinoma.



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Ewing's Sarcoma of Bone

Plate 10

Fig. 9. Photograph showing sagittally cut surface of the femur of which Figure 1 is a preoperative clinical roentgenograph. This picture shows clearly that the extent of tumorous involvement of the bone is far greater than could have been suspected from the preoperative roentgenograph. Of note are the nodules of neoplastic tissue at the upper end of the major marrow cavity.

Fig. 10. Roentgenograph of a thin slice of the bone, including the lower end, cut in the sagittal plane from the femur illustrated in Figure 9. Even here the actual extent of the involvement is not reflected roentgenographically.

Fig. 11. Photograph of the cut surface of the affected innominate bone, and the neoplastic tissue adherent to it, from the case illustrated in Figure 3. The bone is cut in the longitudinal plane, through the region of the iliac fossa. The iliac portion of the bone has been largely destroyed and replaced by a tumor mass visible to the left, while on the right is the tumor mass which extended into the pelvis.

Fig. 12. Photograph showing extensive tumorous involvement of part of the vertebral column in the case also illustrated in Figures 1, 9, and 10.

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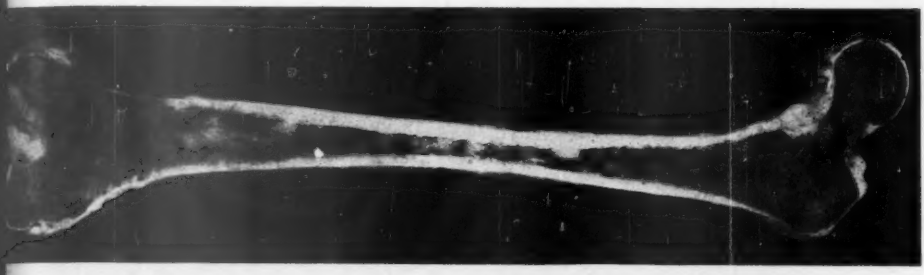

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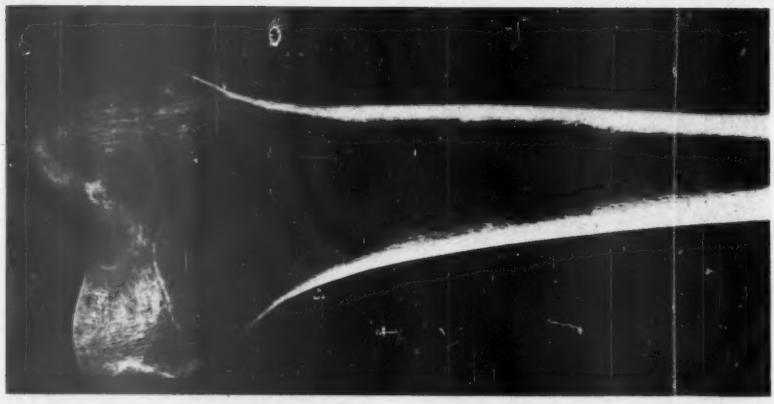

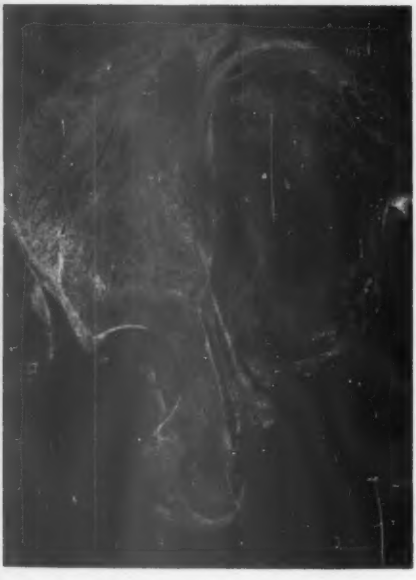

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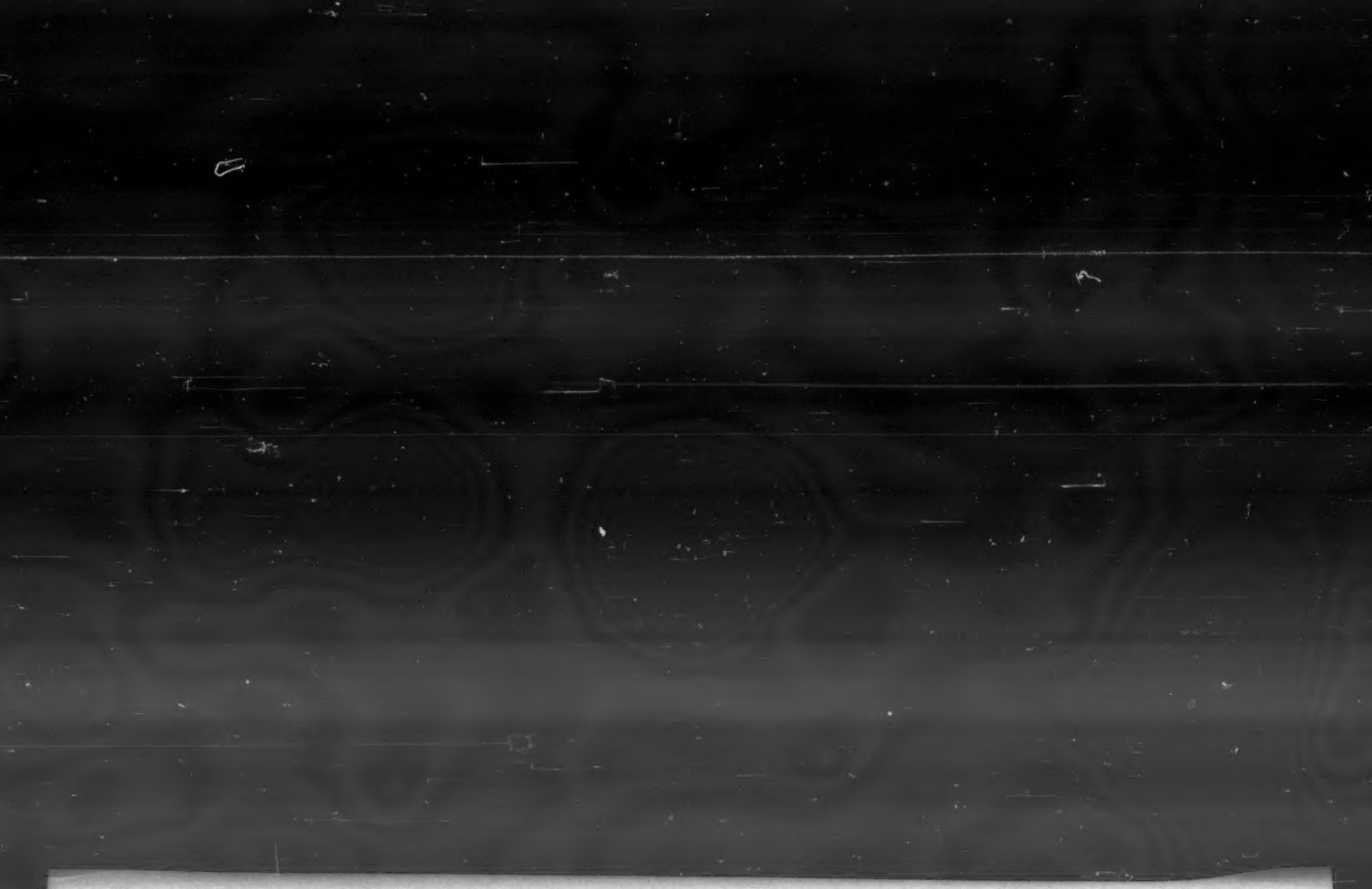
PLATE 11

FIG. 13. Photomicrograph showing the general cytologic pattern in a cellular and relatively unmodified area of a Ewing's sarcoma. Neoplastic tissue is poor in blood vessels and the nuclei are enmeshed in a loose and more or less vacuolated cytoplasmic fabric. The tissue came from the case illustrated also in Figure 7. × 500.

FIG. 14. Photomicrograph showing the general cytologic pattern in a cellular and relatively unmodified area from a Ewing's sarcoma in another case. In this lesion, the nuclei are crowded together and the cell boundaries are not distinct. The tissue came from a male, 18 years old, in whom the presenting lesion was in a fibula and who died 3½ years after the onset of his complaints. × 500.

FIG. 15. Photomicrograph showing the cytologic pattern presented by most of the neoplastic tissue removed for biopsy, in the case illustrated also in Figure 4. From the tissue illustrated, in which small, dark, hyperchromatic nuclei are intermingled with leukocytes, it would not have been possible to tell that one was dealing with a tumor, much less a Ewing's sarcoma. It was only after many sections had been prepared from all the tissue submitted that a few microscopic fields of well preserved neoplastic tissue were found, permitting a diagnosis. × 250.

FIG. 16. Photomicrograph showing the cytologic picture presented by fields of grossly hemorrhagic neoplastic tissue in a case of Ewing's sarcoma in a male of 19 years, whose presenting lesion was in an iliac bone. It was only in the areas in which the neoplastic tissue was hemorrhagic and necrotic that one noted the pattern of tumor cells collaring capillary or larger vascular spaces. The tumor cells themselves do not make up the walls of the vascular spaces. × 250.



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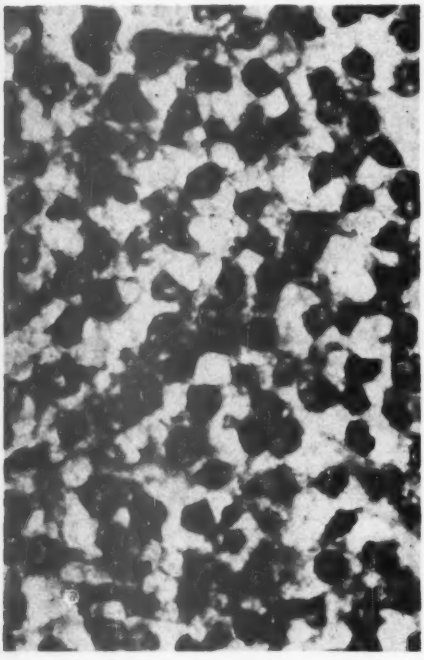

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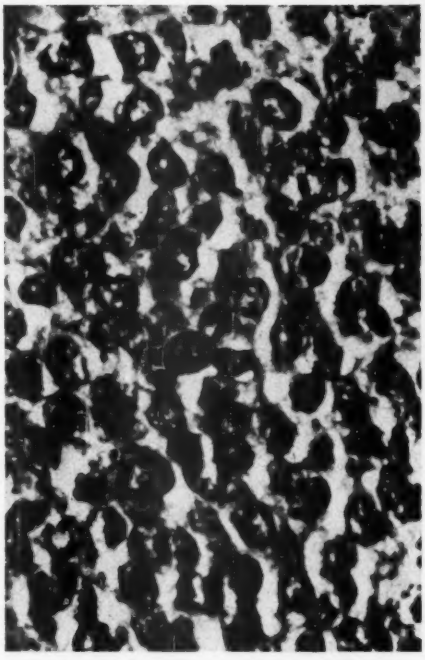

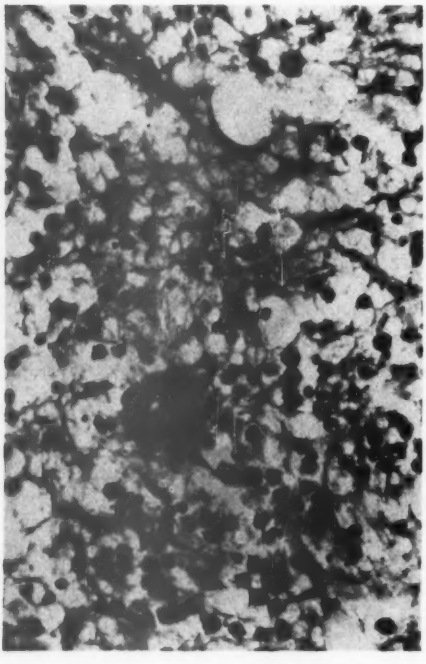

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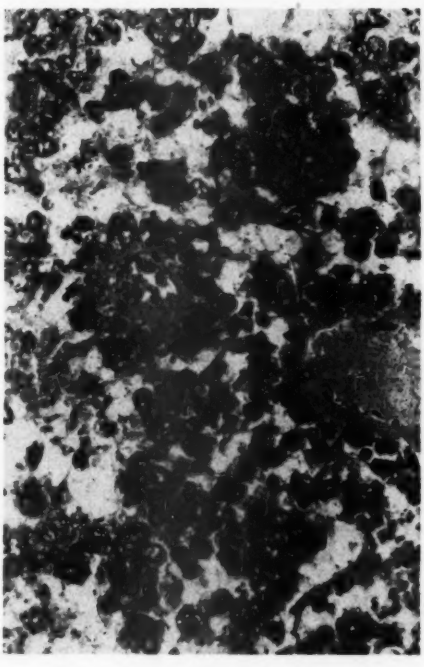

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Plate 12

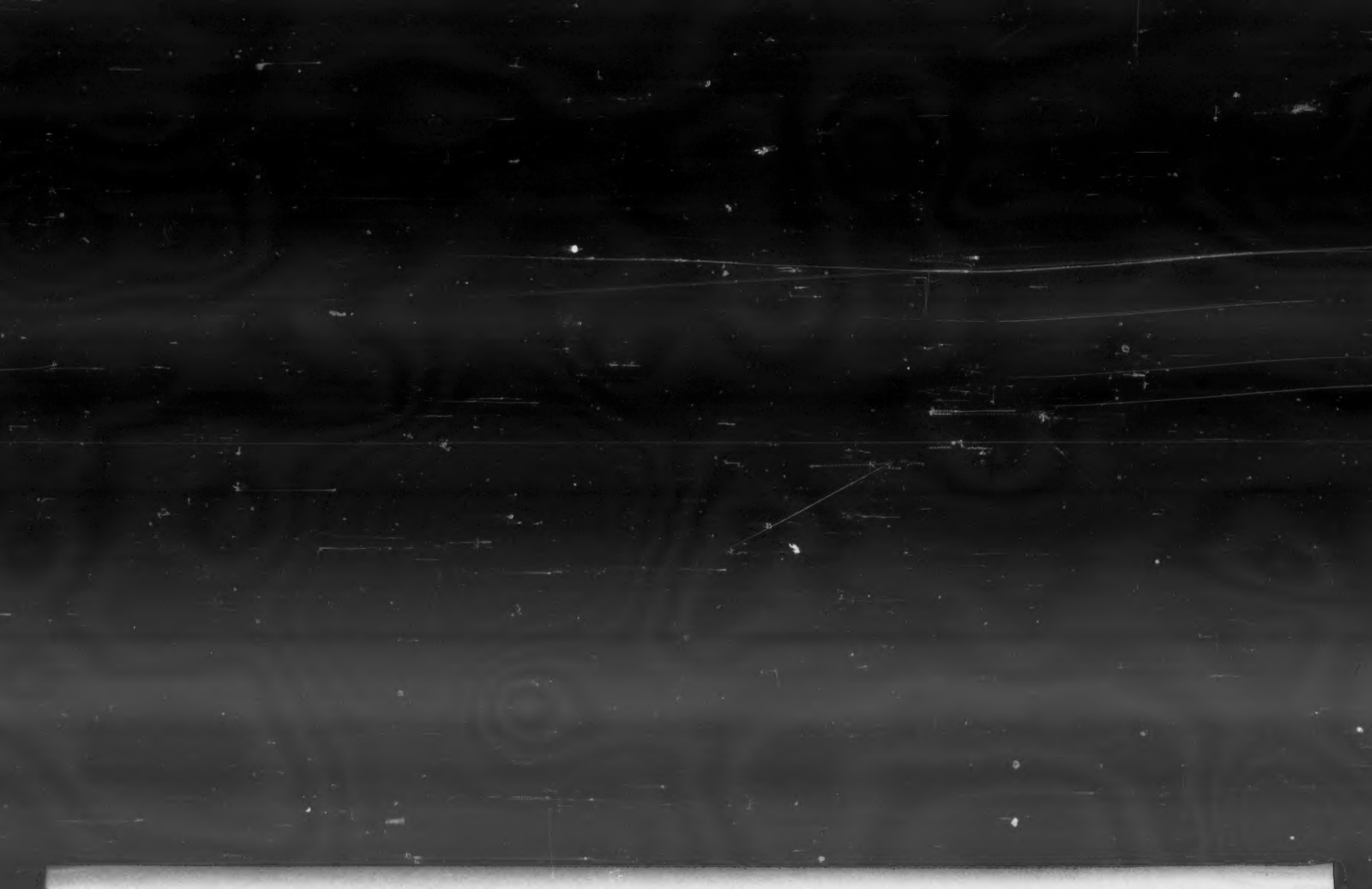
Fig. 17. Photomicrograph showing the general cytologic pattern of a tumor field in a metastatic lesion of Ewing's sarcoma in a rib in the case illustrated also in Figures 1, 9, and 12. As a result of cell degeneration in some places, there are suggestions of ring-like formations. (For comparison with Fig. 18.) × 500.

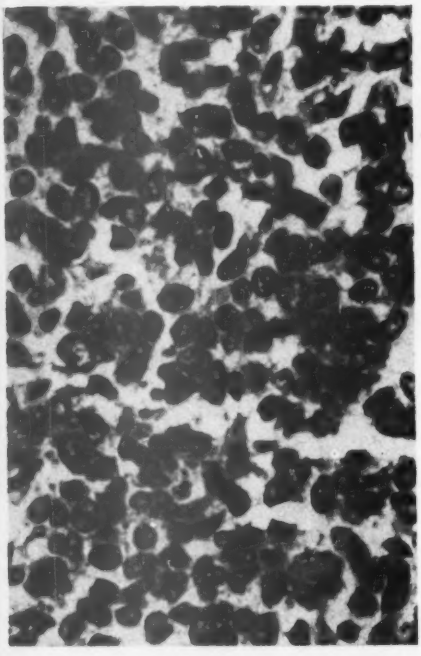
Fig. 18. Photomicrograph showing the general cytologic pattern of a Ewing's sarcoma in a fibula of a girl, 4 years of age. The "rosette-like" formations are constituted by rings of viable tumor cells surrounding cores of degenerated tumor cells, the shadows of which are still perceptible. Such spurious rosettes or pseudorosettes may be compared with the genuine ones shown in Figure 21. × 250.

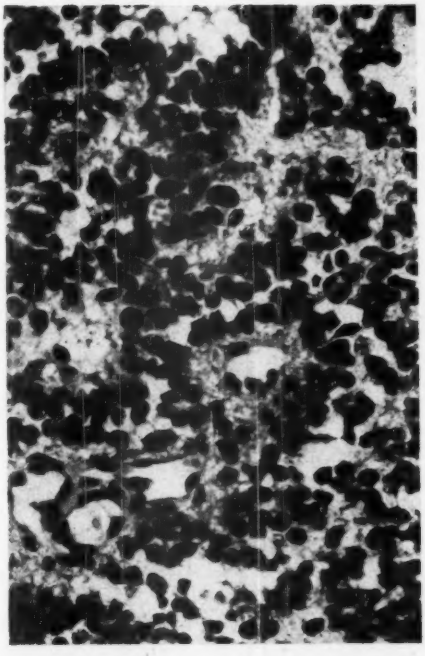
Fig. 19. Roentgenograph showing a rarefying, destructive lesion in the neck of a femur, a lesion which was clinically considered to have an inflammatory basis. One could not tell from this picture that the lesion was actually a metastatic neuroblastoma. The patient was a boy of 3 years whose clinical complaints were pain in the involved hip region and limping of 3 months' duration.

Fig. 20. Photomicrograph showing the general cytologic pattern of the neoplastic tissue curetted from the femoral neck in the case illustrated in Figure 19. No more definitive diagnosis could be made from the curettings than that we were dealing with a malignant tumor. × 500.

Fig. 21. Photomicrograph showing the general cytologic pattern of an involved lymph node removed from the cervical region in the case illustrated in Figures 19 and 20. Of note are the classic rosettes, permitting a definitive diagnosis of sympathicoblastoma in this case. × 500.







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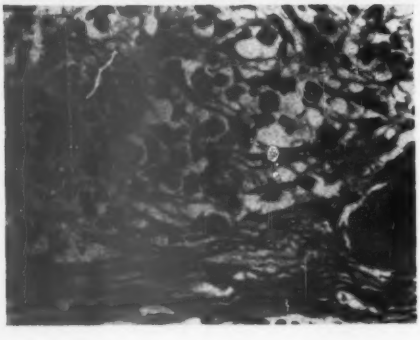

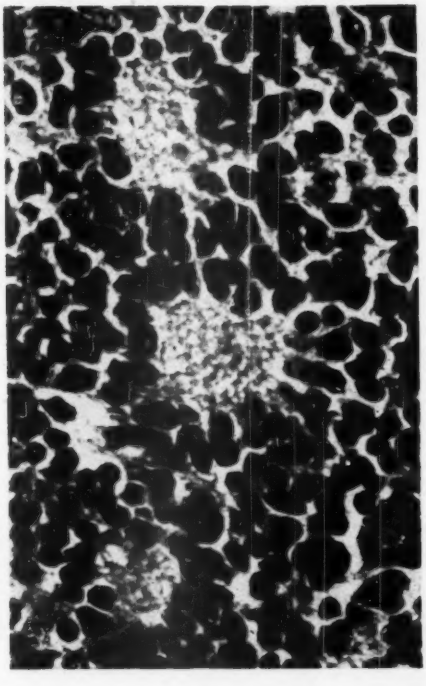

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FATTY INFILTRATION OF THE LIVER IN PATIENTS WITH MONGOLISM AND IN CHILDREN WITH HYDROCEPHALY AND MICROCEPHALY *

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The present investigation was undertaken in connection with a comprehensive study on mongolism carried out by Dr. Clemens E. Benda and his collaborators at the Wallace Research Laboratory of the Wrentham State School.

The pathology of the mongoloid liver has not been described before. In Brousseau's [1] monograph on mongolism in 1928, the liver is not mentioned.

MATERIAL AND METHODS

Forty-three livers of mongoloid patients were examined microscopically. Thirty-four of these came from cases which were studied clinically during life and of which extensive autopsy reports existed. The diagnosis was made on the grounds of a thorough examination of the many stigmata of mongolism. The endocrine glands of most cases were examined by Dr. Benda and his results are published elsewhere.[2]

Almost all of the material was fixed in 10 per cent formalin, which was neutralized in about half of the cases. Unfortunately, very little material was available for glycogen stains. The examination for glycogen appears very desirable and will be carried out in the future. Frozen and paraffin sections were made of almost all of the livers and the following staining methods were employed: hematoxylin and eosin, sudan IV for fat, Heidenhain's azocarmine-aniline blue or Mallory's triple stain, and Giemsa's stain in a few cases.

The livers of the mongoloid patients were compared with a control series of 49 livers of mentally deficient patients of 1 to 30 years of age (Table III). The control series comprised 35 idiots and 14 imbeciles and morons with varying etiologic factors: developmental and hereditary disorders, birth injuries, post-infectional and post-traumatic states, as well as a few of undetermined origin. The cases were taken from the same group of 170 autopsies of which the mongoloid patients were another part. All those cases were used which were of comparable age and of which sufficient material was available.

RESULTS

The livers of mongoloid patients tend to be small (see Tables I and II). Of 34 livers, 20 were underweight, 8 within normal range, and 6 overweight. Extreme underweight (below 25 per cent) was found in

* Received for publication, December 22, 1945.

10 cases (C 94, C 181, 15, 152, 17, 139, 58, 126, 83, and 71), extreme overweight in 3 cases (C 62, 148, and 65). While the average hepatic weight is considerably below normal standards, it is rather above the average for mentally deficient patients other than mongoloids, as can be seen in Table I.

A connection between the fat content of the livers and their weight cannot be established. Of the 3 livers with a great increase in weight, one had an extreme amount of fat and 2 showed a moderate amount. Of 10 extremely light livers, 5 had a marked degree of fatty deposit, one had a moderate amount, and 4 showed hardly any fat.

Many livers showed a yellowish tinge and on section revealed a light surface, which made one suspect fatty deposits. Usually the micro-

TABLE I

Weights of Livers *

Mongoloids, aged 9 days to 28 years			Mentally deficient patients, exclusive of mongoloids, aged 1 to 30 years	
No. of cases	34		49	
Cases normal or ± 5% in weight	8	23.5%	6	12.2%
Cases, overweight	6	17.7%	7	14.3%
Cases, underweight	20	58.8%	36	73.5%
Cases of extreme underweight, below 25%	10	29.4%	20	40.8%
Cases below 50%	0	0	5	10.2%

* For standard values the table of Coppoletta and Wolbach was used (Coppoletta, J. M., and Wolbach, S. B. Body length and organ weight of infants and children. *Am. J. Path.*, 1933, 9, 55–70.)

scopic examination revealed far more fat to be present than was estimated at autopsy. Only one liver (case 148) was found to be actually oozing fat and this liver was also the only one that floated on water. All livers showed a smooth surface. Sometimes increased lobular markings were seen, suggestive of an increase in fibrous tissue. In general the macroscopic appearance of the livers of mongoloid patients is not characteristic.

The main pathologic features are presented in condensed form in Table II. The two outstanding points are: the clear division into two age groups, above and below 2 years, which differ in their essential features; the great frequency of pathologic amounts of fat in the older age group, often accompanied by a moderate increase in fibrous tissue.

Fatty Vacuolization

Twenty-five of the 31 livers from patients more than 2 years of age contained a distinctly abnormal amount of fat. The character of these fatty deposits was clearly recognized in the most severe cases (++++),

Condensed Chart of the Findings in the Livers of Mongoloid Patients

Case no.	Sex	Age	Weight in gm.	Fat	Fibrosis	Congestion	Tuberculosis of liver†	Cause of death
*C 76	F	9 days	90	−	−	++	−	?
*C 94	F	6 wks.	76	−	−	++++	−	?
*C 162	M	2 mos.	?	−	−	+	−	?
*C 181	M	2½ mos.	75	−	−	+++	−	?
*C 95	M	4½ mos.	?	+	+	−	−	?
*C 62	F	6 mos.	260	++	++	++	−	?
120	M	7 mos.	240	+	+	++	−	Lobar pneumonia
15	M	7½ mos.	160	−	+	+++	−	Multiple abscesses
152	M	8 mos.	190	+	−	++	−	Scarlet fever, bronchopneumonia
154	M	1 6/12 yrs.	290	+	+	−	−	Meningitis
140	M	1 8/12 yrs.	?	++	++	++	−	Bronchopneumonia
102	M	1 10/12 yrs.	390	−	−	−	+	Tuberculous meningitis
*C 102	F	2 4/12 yrs.	?	+++	++	++	−	?
99	M	4 5/12 yrs.	570	+++	+	−	−	Brain edema
57	F	4 5/12 yrs.	450	+++	++	−	−	Septic sinus thrombosis
103	M	6 7/12 yrs.	680	++	++	+++	−	Lung abscess
124	M	7 yrs.	640	+++	+++	−	−	Lung embolism
118	M	7 3/12 yrs.	605	++	−	++	+	Tb. of lungs
47	F	8 7/12 yrs.	800	+	++	−	−	Diphtheria, pneumonia
25	M	8 8/12 yrs.	?	++	+	−	−	Bronchopneumonia
125	M	8 9/12 yrs.	670	−	++	+++	+	Tb. of lungs
134	F	9 7/12 yrs.	710	+	−	+	−	Intussusception
17	F	9 8/12 yrs.	460	+++	++	+++	−	Bronchopneumonia
141	M	10 4/12 yrs.	740	++++	+	−	+	Tb. of lungs
139	M	11 1/12 yrs.	690	+++	++	+	−	Bronchopneumonia
133	F	12 yrs.	?	++	++	++	−	Volvulus
82	F	13 10/12 yrs.	890	+++	−	−	+	Tb. of lungs
155	M	14 4/12 yrs.	?	++	−	−	+	Tb. of lungs
32	M	14 11/12 yrs.	830	+++	+	−	−	Bronchopneumonia
116	F	15 4/12 yrs.	1100	+	−	−	−	Tb. (miliary)
100	F	15 8/12 yrs.	1135	+++	++	++	+	Tb. of lungs
148	M	15 10/12 yrs.	1650	++++	+	−	+	Tb. of lungs
85	M	16 yrs.	1400	+++	+++	++	−	Status epilepticus
58	M	16 3/12 yrs.	880	+++	++	++	+	Tb. of lungs
81	M	17 yrs.	1140	++++	++	−	+	Tb. of lungs
90	M	17 yrs.	1590	−	−	−	−	Gangrene of lung
126	F	17 1/12 yrs.	1050	+++	+++	−	−	Tb. of lungs
83	M	17 9/12 yrs.	880	+++	+	++++	+	Tb. of lungs
65	M	18 1/12 yrs.	2100	++	++++	+	−	Tb. of lungs
146	M	18 4/12 yrs.	1510	++	++	−	−	Tb. of lungs
*M 3	F	20 yrs.	?	+	+++	+++	+	Tb.
22	M	20 6/12 yrs.	?	+++	++	++	−	Bronchopneumonia
71	F	28 5/12 yrs.	820	++	−	++	+	Tb. of lungs

\+ = Slight degree, probably not pathological.
++ = Definitely pathological, but not pronounced.
+++ = Marked degree.
++++ = Extreme degree throughout organ.

* Material from these cases was contributed by other hospitals.

† The presence of tuberculous foci is marked with a cross regardless of the degree of involvement.

in which all liver cells were filled with fat droplets up to 50 μ in diameter (Figs. 2 and 3). These extreme cases still showed the lobular pattern. The liver cords radiating from the central veins were not disrupted. There was, in addition, a considerable amount of cytoplasm between the fat droplets, which showed no degenerative signs. The impression was that of fatty "infiltration" rather than of "degeneration," and this also held true for most of the less extreme cases. Typ-

TABLE III

Comparative Pathology of the Liver

	Mongoloids, aged 1 to 28 years			Mentally deficient patients, exclusive of mongoloids, aged 1 to 30 years	
No. of cases	34			49	
Cases with tuberculosis	17	50.0%		19	38.8%
Cases without fat or with small amounts of fat (+ or −)	8	23.5%		29	59.2%
Cases with fat (++ to ++++)	26	76.5%		19	38.8%
Cases with fat, with tuberculosis	13	76.5%	of cases with tuberculosis	8	42.1%
Cases with fat, without tuberculosis	13	76.5%	of cases without tuberculosis	11	36.7%
Cases with much fat (+++ to ++++)	17	50.0%		10	20.4%
Cases with much fat, with tuberculosis	8	47.1%	of cases with tuberculosis	3	15.8%
Cases with much fat, without tuberculosis	9	52.9%	of cases without tuberculosis	7	23.3%
Cases with fibrosis (++ to ++++)	19	55.9%		6	12.3%

ically, the fat was accumulated around the portal vessels and little was found near the central veins (Fig. 1), but 5 cases definitely showed the opposite pattern—a distinct pericentral accumulation. In 2 instances there was a considerable increase in fat droplets around the central veins as well as around the portal vessels, leaving the intermediate zones of lobules free from fat. In the extreme cases, the fat filled the liver completely and uniformly. In one case (no. 140) the Kupffer cells were almost all filled with fat in very fine granules in addition to a moderately severe fat deposit in the liver cells which showed no other unusual features (Fig. 7).

A remarkable distribution of fat was seen in case 124 in which all arteries and arterioles were very thick-walled and partly occluded. The parenchyma was filled quite uniformly with fatty vacuoles of

varying sizes, but the liver cells directly adjacent to the affected vessels contained very little fat. The vessels appeared lined on the outside by one layer of almost normal liver cells (Fig. 8).

Of 25 nonmongoloid mentally deficient children under 15 years of age, 11 showed fat in the liver and in 8 the pathologic picture was not essentially different from that found in mongolism. In all there was hydrocephalus or microcephalus with definite signs of brain pressure. A description and discussion of the 5 most extreme cases will be given later in this paper.

Fibrosis

An increase in the fibrous tissue occurred less frequently than did fatty vacuolation (18 of 31 cases over 2 years of age). The increase was always predominantly periportal, even if the fat was accumulated pericentrally. The outstanding fact is that the proliferation usually remained moderate in degree and showed very little tendency to progress into the lobules at the expense of the parenchyma. Nodular cirrhosis was never found. Typically, the capsule was thickened to about twice its normal size, but was even and smooth, and the periportal tissue appeared increased with thickened septa dividing the lobules. Only one liver (no. 65) showed a very high degree of fibrosis but even its surface was smooth and replacement of liver cells by fibrous tissue was moderate. Thirteen of 26 cases above 7 years of age showed no other abnormalities but fatty deposits and some fibrosis.

Degenerative Phenomena and Congestion

At least half of the cases presented degenerative phenomena of varying degrees in the liver cells. In most cases the degeneration was not uniform and consisted only of swelling of the cells. Necrosis and nuclear degeneration were infrequent. When they occurred, big fat vacuoles were often seen in the affected cells (Fig. 4). One case (no. 125) showed a high degree of necrosis throughout the liver without any indication of regenerative activity. This liver was not typical of mongolism, as there was no fat detectable in the cells. The patient was a Negro, and some doubt is thrown on the diagnosis through the fact that his pituitary and thyroid glands were also not typical of mongolism.

Congestion is found in connection with so many diseases and is so often connected with terminal circulatory failure, either local or general, that it was not surprising to find it in almost half of the cases. It was almost always confined to those areas which did not show fatty vacuolation (Fig. 5), but in 2 cases the opposite distribution was observed.

Congestion was a remarkable feature in some cases of the age group

below 2 years. Nine of the 12 livers in this group showed a considerable amount, and 3 showed engorgement of a severe degree accompanied by pericentral hemorrhagic necrosis. In these cases the liver cords were greatly compressed by the engorged sinusoids (Fig. 6).

In general, it appears that degenerative phenomena and congestion occur secondarily in livers which are primarily fatty. The obviously abnormal metabolism probably predisposes the liver tissue to subsequent disease. The existence of cases which show extreme amounts of fat without apparent degeneration makes it probable that the variation in the degenerative signs observed was due to the action of infectious diseases, chiefly tuberculosis, on the abnormal liver.

Among the 31 livers of mongoloid patients who were 2 years or more of age, there was one which was essentially normal (no. 90). It is interesting to note that this case was regarded clinically as "borderline." The weight of the brain was the only normal weight in the series and the gonads were better developed than is usual in a mongoloid patient.

Discussion

Fatty livers occur most frequently in chronic alcoholism, in tuberculosis, and in general obesity. The last two of these conditions must be considered here. Half of the mongoloid patients died of tuberculosis and the liver was involved in 76.5 per cent of these cases. But a closer study of Tables II and III reveals that the fatty deposits occurred without relation to the presence or absence of tuberculosis. In the first place, fat occurred with exactly the same frequency, 76.5 per cent, in those cases which had no tuberculosis. In Table II this is most easily seen when attention is paid to the age group between 2 and 10 years, in which tuberculosis as the cause of death occurred only twice in 11 cases. However, 8 cases showed abnormal amounts of fat in the liver. In one of the 2 tuberculous patients no fat was found.

Furthermore, a comparison of the figures in Table III demonstrates the same fact in the control series: the incidence of fatty livers in cases with tuberculosis was not different from the incidence in the series as a whole, including the cases without tuberculosis. Tuberculosis as a cause of death was slightly less frequent (38.8 per cent) than in the mongoloid patients (50 per cent). Fat in the livers occurred only half as frequently (38.8 per cent compared with 76.5 per cent), but the incidence in cases with tuberculosis (42.1 per cent) differed little from that in cases without tuberculosis (36.7 per cent). It can, therefore, be concluded that the rôle which tuberculosis plays in producing fatty livers in the mongoloid patient can only be slight and that some other explanation must be found for the phenomenon. The fact that the

same is true for mentally deficient patients other than mongoloids actually raises some doubt as to the mechanism involved in the origin of fatty livers in tuberculous cases. It appears desirable to reinvestigate this problem in a large series of material.

Whether the fatty livers in mongoloid patients may be due to general obesity is a more difficult problem. Obesity as such is not an etiologic entity. It may arise through a predominantly fatty diet as well as through a variety of metabolic disorders. The patients investigated in this paper all received a well balanced diet. The obesity of the individuals varied a great deal and no connection could be found between the degree of obesity and the fattiness of the liver. Many patients with extremely fatty livers were not at all obese. However, the average relative weight of the mongoloid is high from the third year of life on (for tables on the weight and length of mongoloid children see Benda [3]). The mongoloid infant is underweight, often very much so; the gradual change to overweight takes place during the second year of life. Fat appears in the livers at about the same time. Both events seem to be consequences of a metabolic disorder.

When dogs are deprived of both the pituitary and thyroid glands, the liver shows a picture very similar to that found in the mongoloid patient.[4] Extensive fatty deposits are seen, often followed by periportal fibrosis. When the thyroid alone is removed, the fatty changes are far less pronounced. Hypophysectomy alone produces a picture of hepatic cirrhosis in dogs. In many cases this is accompanied by fatty deposits (Graef, Negrin, and Page [5]). These authors believe that the infundibulum and the stalk, rather than the pituitary gland, exert an influence on the fat metabolism of the liver. The picture which they described resembles that of the liver of mongoloids in some respects, but the analogy is not nearly as close as in the dogs which were deprived of both the pituitary and thyroid glands. In particular, the great amount of fibrosis leading to a typical nodular cirrhosis with atrophy of liver cords was never observed in mongoloids. It is, of course, questionable whether the dog and man react alike to a deficiency of their endocrine glands. Results seem to point to a greater tendency of the dog's liver to react with severe proliferation of fibrous tissue. Dogs subjected to prolonged feeding of a high-fat diet first showed fat deposits in the livers; later a typical cirrhosis developed.[6] In the mongoloid, fatty infiltration is the rule and fibrosis often follows, but reaches only a moderate degree, while the fat may increase to extreme amounts.

Graef, Negrin, and Page [5] have reported 2 cases of tumor indirectly involving the pars nervosa and the stalk of the pituitary gland, and

showing considerable fatty deposit in the liver with "otherwise normal" lobules. These findings seem to point in the same direction, namely, that in man, deficiency of the pituitary body or its stalk does not cause cirrhosis but predominantly causes a fatty change in the liver. Whether brain pressure plays a part in the mongoloid patient in affecting the pituitary body and hypothalamus, as described by Kraus [7] in certain tumors causing intracranial pressure, is a point of speculation. Morgan [8] has demonstrated defects in the hypothalamus of mongoloids, and intracranial pressure—not spinal fluid pressure—probably exists at some time during the life of the patient with mongolism.[9]

In this connection, it is an interesting fact that there are a number of cases in the control series of mentally deficient patients in which a fatty liver might also be attributed to brain pressure and a damaged infundibulum.

Case 97. 1-year-old male, idiot, microcephalus, died of bronchopneumonia. Not obese. Large old intracranial hemorrhages. Cortex atrophic; weight of brain, 138 gm. Skull small, 12 inches in circumference, sutures firmly united. The liver showed considerable fatty infiltration (+++).

Case 73. 3½-year-old male, imbecile, hydrocephalus of 35 inches in circumference, which was the cause of death. 8,000 cc. of spinal fluid was removed. At autopsy, body in fair state of nutrition. The liver showed extreme amounts of fat (++++).

Case 40. 5-year-old female, idiot, microcephalus. Cause of death: status epilepticus. Small and thin child. Blind. Head circumference, 17¼ inches. Hydrocephalus internus. Widened infundibulum. Liver underweight, fatty (+++).

Case 98. 13-year-old female, idiot, birth injury. Very obese. Cause of death: cystic brain. Skull thickened. Brain weight, 498 gm. Cystic degeneration of right and left occipital, and right parietal and temporal lobes. Liver weight, 820 gm.; much fatty infiltration (+++).

Case 64. 14-year-old female, idiot, hydrocephalus. Cause of death: status epilepticus. Well nourished. Brain weight, 505 gm. Extreme internal hydrocephalus, infundibulum enlarged to an opening of 3 cm. depth and 1.5 cm. width. Liver weight, 640 gm. Severe fatty infiltration (+++).

In all of these 5 cases the cause of the fatty infiltration in the liver was not obvious, but it appears remarkable that they should all have brain damage of a kind which produces brain pressure. In case 40 and case 64, the infundibulum was found to be severely damaged by pressure.

Benda [2, 10, 11] has described the pathology of the pituitary gland in mongoloids and has accumulated evidence that hypofunction of the pituitary gland is an important factor in mongolism. The picture of the fatty liver as described above is consistent with this glandular deficiency. However, it is not possible, at the present time, to say definitely whether the pituitary gland, itself, or the infundibulum and stalk may be responsible for the fatty infiltration, as all three have been shown to be involved.

The liver of the infant mongoloid does not show the typical changes observed in the older child and adult. The endocrine balance of the infant is obviously different (for instance, he is usually much underweight) and the difference is reflected in the livers. One-third of the livers from patients under 2 years of age are found to be essentially normal. Two-thirds show congestion which is often extreme. It has been found that dogs deprived of their adrenals show great vascular congestion and hemorrhages around the central veins of the liver.[12] Benda [2] has investigated the pathology of the adrenal glands of mongoloids and has shown that characteristic features of the infant mongoloid (low temperature, fatigue, etc.) may be due to adrenal insufficiency. It seems possible, therefore, that the livers in the young age group reflect this insufficiency. Obviously, the pituitary factor is not dominating at this early age.

Summary

Forty-three livers of mongoloid patients showed a considerable degree of fatty deposit in the majority of cases, often accompanied by a moderate fibrosis. The incidence of fatty livers was about 63 per cent. For patients aged 1 to 28 the incidence of fatty livers was twice as high as in a control series of other mentally deficient patients. It was shown that the fatty deposits are not connected with the occurrence of tuberculosis nor with the degree of obesity in the individual. However, they arise during the second year of life, at the time when the average relative weight of the mongoloid child rises, under the influence of a metabolic disorder. The pathologic picture of fatty infiltration and fibrosis is consistent with hypopituitarism and hypothyroidism which are characteristic for the mongoloid patient.

The mongoloid infant often shows an extremely engorged liver with hemorrhagic necrosis, which may be connected with an adrenal insufficiency.

Fatty infiltration of the same type was found also in several children of a control series of mentally deficient patients. The fatty deposits

were again unrelated to the presence of tuberculosis or obesity. However, in these cases, certain brain lesions, hydrocephalus, microcephalus, and cystic disease, which had produced brain pressure, were found.

I am greatly indebted to Dr. Benda, whose work and helpful advice made this contribution possible.

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DESCRIPTION OF PLATES

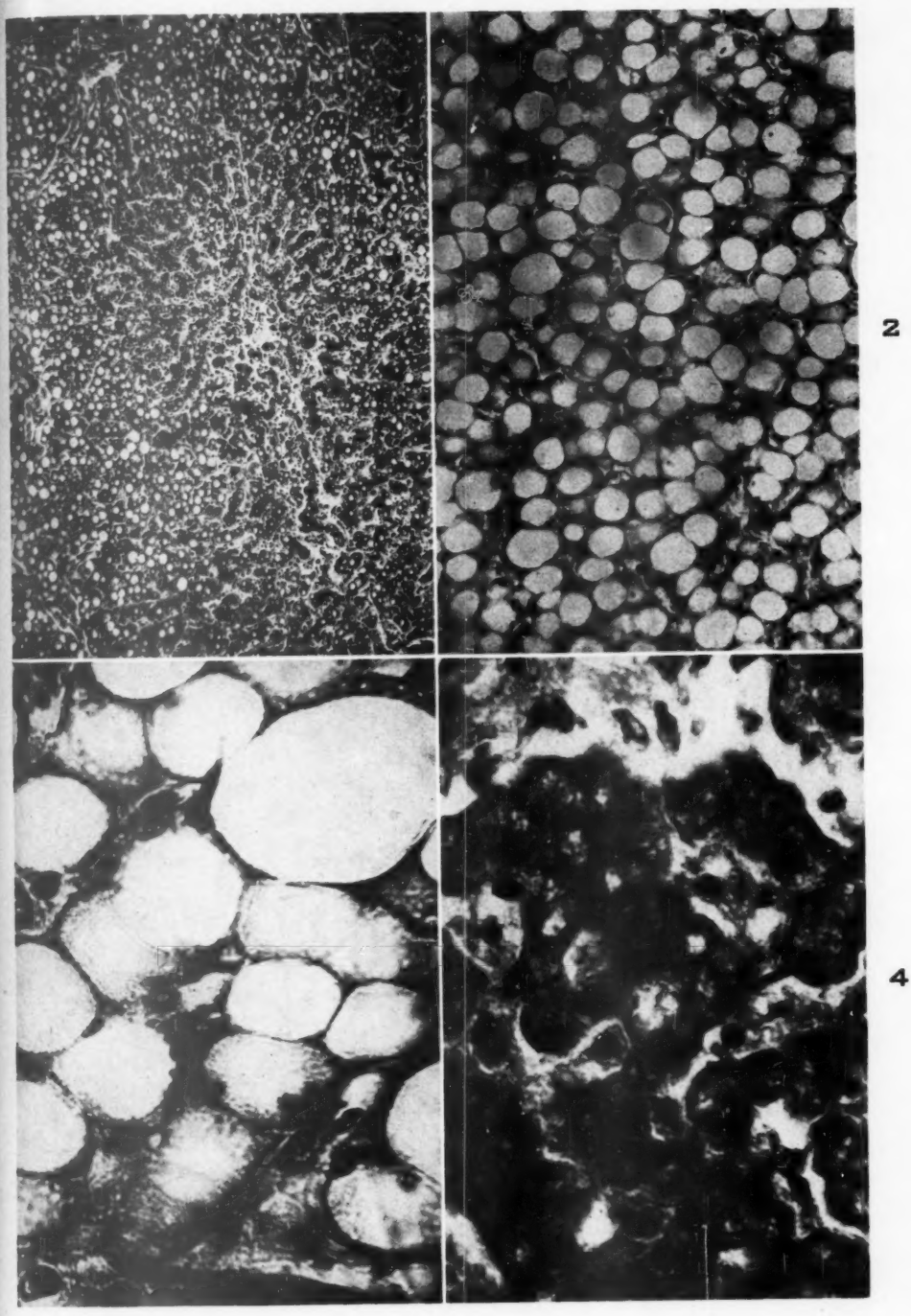
PLATE 13

FIG. 1. Case 139, 11 years of age. Fatty vacuolation, most severe in the periphery of the lobule. Moderate fibrosis. Hematoxylin and eosin stain. × 95.

FIG. 2. Case 148, 16 years old. Extreme fatty vacuolation, filling the liver uniformly. Heidenhain's azocarmine-aniline blue stain. × 280.

FIG. 3. From the same case as Figure 2. × 890.

FIG. 4. Case 83, 18 years of age. Cell degeneration. Fatty vacuoles in dissolution. Fibrosis is slight and confined to the periportal areas (upper part of field). Hematoxylin and eosin stain. × 740.



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Roosen-Runge Liver in Patients with Mongolism

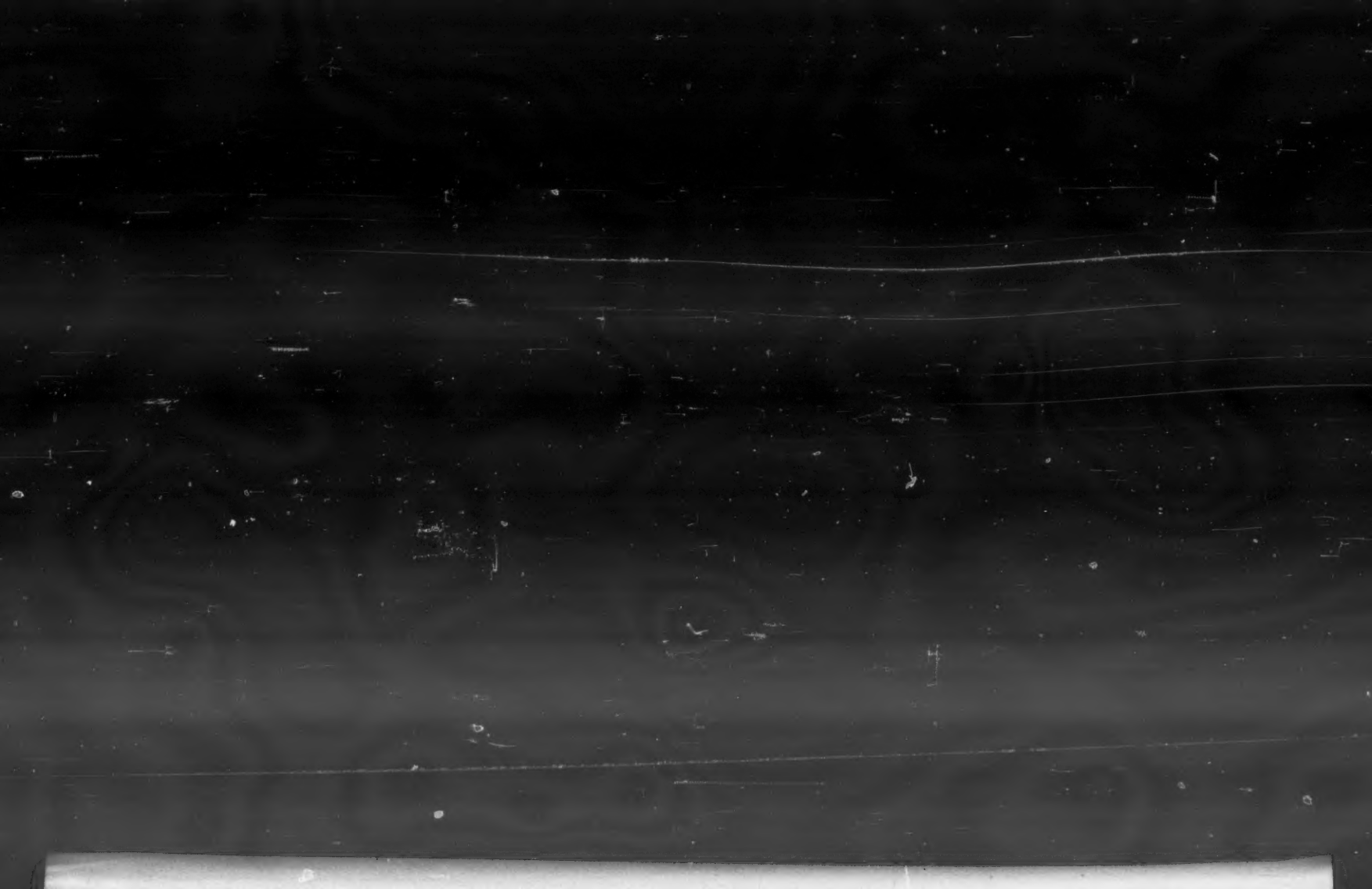
PLATE 14

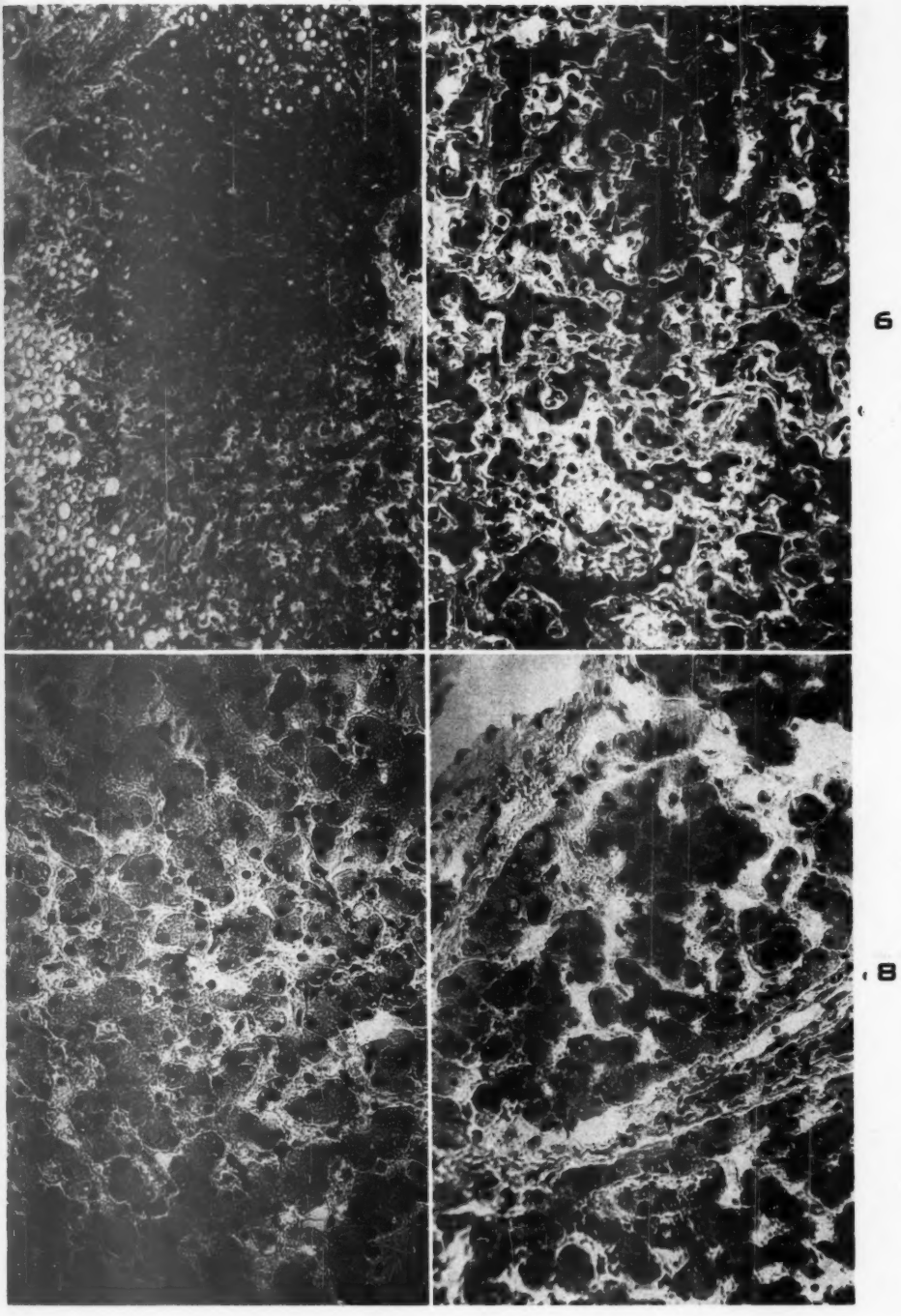
FIG. 5. Case 58, 16 years old. Pericentral engorgement, periportal vacuolation. Mallory's triple stain. × 130.

FIG. 6. Case C-181, 2½ months of age. Engorgement of all sinusoids. The liver cords are compressed and shrunken. A few of the very rare, fatty vacuoles are seen in the field. Hematoxylin and eosin stain. × 160.

FIG. 7. Case 140, 1 year, 8 months of age. The Kupffer cells are filled with fat (black in the photomicrograph). In the area selected, the liver cells contain little fat. Sudan IV stain. × 240.

FIG. 8. Case 124, 7 years of age. The liver cells contain much fat, except for a one-celled layer which accompanies the vessels and the fibrous tissue around them. The condition of the vessels suggested an old periarteritis nodosa. Sudan IV stain. × 240.





HISTOPATHOLOGY OF MONOCYTIC LEUKEMIA *

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Monocytic leukemia emerged as a distinct entity in 1913 when Reschad and Schilling-Torgau [1] reported the first case. Subsequent medical literature contains an increasing number of case reports and several comprehensive reviews on the subject.[2-8] Most of the publications disclose a detailed account of the clinical manifestations, have excellent descriptions and photomicrographs of monocytic cells as they appear in blood smears, and contain lengthy discussions on the origin of the monocyte. In contrast, however, the histopathologic changes have been reported in detail only sporadically, and in none of the papers in the English language have these descriptions been supported by adequate illustrations. For this reason we are recording the following 8 cases that have been necropsied at the Jefferson Medical College Hospital in the past 7 years.

SUMMARY OF CLINICAL MANIFESTATIONS

Case 1

A white boy, 16 years of age, developed headache 9 days before admission and fever 4 days later following typhoid inoculation. He entered the hospital because of soreness of the teeth and gums. He had oppression in the chest, mass in the left side of the neck, enlarged axillary lymph nodes and spleen, and hypertrophied gums. Studies of the blood showed 2,200,000 erythrocytes per cmm., 64 per cent hemoglobin, and 2,700 leukocytes per cmm. with 64 per cent blast cells. Fever was uncontrolled, leukocytes increased to 210,000 per cmm. and consisted of monocytes and monoblasts, and the bone marrow showed monoblastic hyperplasia. The patient died 26 days after the onset of symptoms.

Case 2

A white man, 30 years old, was well until 8 weeks before admission, during which time he had lost 27 lbs. in weight and developed a dry cough, bleeding gums, and an enlarged spleen. A blood count showed 1,400,000 erythrocytes per cmm., 36 per cent hemoglobin, 43,000 leukocytes per cmm., of which 64 per cent were monoblasts and 5 per cent monocytes, and 44,000 platelets per cmm. Subsequently, the leukocytes increased to 177,000 with 90 per cent monoblasts. Smears of sternal marrow disclosed a hyperplasia of monoblasts, many of which contained Auer's bodies. The patient died 4 months after the onset of symptoms.

Case 3

A 35-year-old white man had a pilonidal sinus removed 4 years previously. This had never healed. He first entered the hospital because of weakness, loss of weight, and pallor. A blood count disclosed 1,200,000 erythrocytes per cmm., 31 per cent

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hemoglobin, 1,400 leukocytes per cmm. with 50 per cent lymphocytes, 4 per cent monocytes, and 12 per cent monoblasts. Smears of sternal marrow disclosed oxidase-negative, monocytoid, primitive cells. Five months later he was admitted a third time because of an infection in the upper jaw. The liver was enlarged and there were changes seen roentgenologically in the right femur. The leukocyte count never rose above 2,000 per cmm. Before death there were 41 per cent monocytes and 12 per cent monoblasts in the peripheral blood stream. The duration of illness was approximately 8 months.

Case 4

A white man, 40 years old, had symptoms of peptic ulcer for several years. He was admitted to the hospital in coma with a history of a sore, bleeding mouth and black and blue spots in the skin of 1 week's duration. Examination disclosed bloody spinal fluid, 3,800,000 erythrocytes per cmm. of blood, 79 per cent hemoglobin, 42,000 leukocytes per cmm., of which 20 per cent were monocytes and 68 per cent monoblasts, 22,000 platelets per cmm., and a high proportion of monocytes and monoblasts in the sternal marrow. The patient died 8 days after the onset of acute symptoms.

Case 5

A white man, 43 years old, entered the hospital because of a painful swelling of the left jaw of 1 month's duration. Two weeks previously he had had 9 teeth removed. A blood count showed 3,600,000 erythrocytes per cmm., 69 per cent hemoglobin, and 9,950 leukocytes per cmm. with 53 per cent monoblasts and 5 per cent monocytes. Later the leukocytes increased to 40,000 per cmm. with 77 per cent monoblasts. The gums became hypertrophied, the liver enlarged, and the patient died 2 months after removal of the teeth.

Case 6

A white man, 57 years old, was well until 5 months before admission when he developed a series of furuncles on the face, neck, and abdomen. In the peripheral blood there were (per cmm.) 2,900,000 erythrocytes, 28,000 platelets, and 3,000 leukocytes, of which 5 per cent were monocytes and 4 per cent monoblasts. The sternal marrow showed an increase in monoblasts, some of which contained Auer's bodies. Subsequently, the leukocytes increased to 94,000 with 68 per cent monoblasts and the patient developed slight hypertrophy of the gums. He died 1 year after the first onset of furunculosis.

Case 7

A white man, 62 years old, entered the hospital because of pain in the hips of 6 weeks' duration and a red to purple rash of 2 weeks' duration. A blood count showed 1,800,000 erythrocytes per cmm., 36 per cent hemoglobin, 84,000 platelets per cmm., and 14,200 leukocytes per cmm., with 28 per cent monocytes and 36 per cent monoblasts. The leukocyte count increased to as much as 141,000 per cmm., with 69 per cent monocytes. The sternal marrow showed hyperplasia of primitive cells that were probably monoblasts. The patient died 12 weeks after the onset of pain in the hips.

Case 8

A white man, 70 years old, entered the hospital with anorexia, fatigability and nausea and vomiting, all of which followed an acute upper respiratory infection 6 weeks previously. For 6 years he had had back pain with questionable roentgenologic evidence of lumbar arthritis. Examination disclosed an enlarged spleen, generalized twitching of muscles, and spasm of the muscles of the legs. There were 2,900,000 erythrocytes per cmm. of blood, 54,000 platelets per cmm., and 4,000

leukocytes per cmm., with 7 per cent monocytes and 25 per cent monoblasts. The leukocyte count never rose beyond 6,300 cells per cmm. The patient died in coma 8 weeks after the onset of symptoms.

Gross Pathologic Observations

Skin. Cutaneous lesions were present in all cases except 3 and 8. Scattered or innumerable petechiae from pin-point to 2 mm. in diameter, with no predilection for any particular part of the body, were present in all of the positive cases. Usually these were flat and not accompanied by any surrounding induration, but in case 7 the immediately subjacent tissue contained definite firm, gray foci measuring 1 to 2 mm. in diameter. In addition to the petechiae, case 1 disclosed numerous papules measuring 2 to 5 mm. in diameter and raised 1 to 2 mm. above the surrounding surface of skin. Intracutaneous and subcutaneous hemorrhages measuring as much as 14 cm. in diameter were present in cases 1 and 7. Centrally, these were slightly elevated but peripherally they merged gradually with the adjacent normal structures.

Mouth. In cases 2 and 4 the mucous membranes of the mouth, and particularly the gingivae, were covered with bright red and recently coagulated blood. In case 5 there were areas of necrosis without hemorrhage scattered throughout the mouth but unusually prominent over the gums. The gingivae in case 1 were hypertrophied to such a degree that in some areas they completely covered the lateral surfaces of the teeth. Their mucosa was covered with coagulated blood but was not ulcerated and the underlying tissue was moderately firm and pinkish gray. In addition, this case showed numerous petechiae in the mucous membrane of the upper lip and left cheek. Case 3 revealed a superficial erosion of the tip of the tongue and a large ulcer occupying the entire inner portion of the left cheek and the adjacent gums. The floor of the latter contained soft necrotic tissue and the base was reddish gray. There were no oral lesions in cases 6, 7, and 8.

Spleen. Splenomegaly was present in all cases, splenic weights ranging from 270 gm. to 1,290 gm., with an average of 720 gm. The capsules were usually tense, bluish gray, smooth, glistening, and thin. In cases 2, 5, and 8 there was, however, some capsular fibrosis of the central portion of the convex surface, and in cases 2 and 8 these areas were adherent to the diaphragm and anterior abdominal peritoneum. In all cases the edges were rounded. The consistency approached that of liver tissue in cases 1, 2, 4, and 6 (weighing 900, 1,270, 550, and 1,200 gm., respectively), was normal in cases 3 (350 gm.) and 7 (400 gm.), and was considerably softer than normal in cases 5 (270 gm.), and 8 (800 gm.). In all cases the follicular markings were

obscured. The pulp in the firm spleens was deep red and beef-like in appearance, whereas in the soft spleens it was mushy, partially liquefied, and pinkish gray. Depressed, gray to brown infarcts of varying sizes were present in the spleens of cases 1 and 2.

Liver. The liver was slightly or markedly enlarged in all except case 8. Hepatic weights ranged from 1,720 (case 8) to 3,800 gm. (case 6), with an average of 2,480 gm. In all the capsule was smooth, glistening, full or tense, and of normal or decreased thickness. As the organ increased in size the edges became more rounded. All livers were of normal or increased firmness and both on external and cut surfaces they were light or dark reddish brown. Lobular markings were accentuated by thin streaks of gray tissue in half of the cases, while in the remainder they were indistinct. None of the livers showed tumor nodules but in case 2 (weighing 3,000 gm.) there were six abscesses scattered throughout the hepatic parenchyma. They were filled with thick, green, purulent material and measured as much as 1.5 cm. in diameter. The gallbladder and bile ducts were normal.

Bone Marrow. The marrow of the vertebrae was examined in all cases and, in addition, that of the sternum, ribs, and femur in some cases. It was essentially normal in case 8, whereas in the other cases it varied from rather friable, mushy, light reddish brown to firm, dry, pale pink, or gray. In none of the cases was there gross evidence of leukemic penetration of the periosteum, osteolysis, osteosclerosis, or tumor formation.

Lymph Nodes. The lymph nodes were grossly normal in cases 3, 4, and 8. The superficial nodes (cervical, supraclavicular, axillary, and inguinal) were enlarged to as much as 3 cm. in diameter in cases 1, 2, and 6, whereas the deep nodes (mediastinal, hepatic hilar, periaortic, and mesenteric) were enlarged to as much as 5 cm. in diameter in cases 1, 2, 5, 6, and 7. In case 6 the hilar nodes of the liver, measuring 4 cm. in diameter, disclosed some matting, whereas in all other cases the nodes were sharply circumscribed, discrete, well encapsulated, and moderately firm. Cut surfaces were usually homogeneously pinkish gray. In case 7, however, they disclosed minute dark red hemorrhages, and in case 2 several of those at the hilum of the liver contained minute acute abscesses filled with greenish yellow pus. The thymus was enlarged and infiltrated with pinkish gray tissue in cases 1 and 5 and was not identified in the remaining cases.

Gastrointestinal Tract. The gastrointestinal tract was normal in cases 2, 3, and 8. There were petechiae in the serosa of the small intestine in cases 1 and 7, while the mucosa of the stomach and small bowel was congested in case 4. Peyer's patches of the terminal portion

of the ileum were hypertrophied and raised 1 mm. above the surface in cases 1 and 5 and in the latter they were, in addition, superficially ulcerated. The cecum and ascending colon of case 7 disclosed several small submucosal ridges of moderately firm tissue. The perirectal fat tissue in case 6 was indurated and the rectal wall measured 1.5 cm. across. Although the entire wall was infiltrated with gray tissue, the submucosa was proportionately thicker than the remaining layers. The mucosa was thrown into large, firm, pink to red folds, the summits of which were superficially ulcerated. The rectal lumen was reduced to less than 1 cm. in diameter. The large bowel above the constriction was dilated and filled with air and fluid feces. The intestinal tract was otherwise normal.

Kidneys. The kidneys weighed from 100 to 350 gm. with an average of approximately 202 gm. Except for being slightly increased in size, those of cases 3, 4, and 8 were normal. In none was the capsule adherent. In cases 2, 5, and 7 the external and cut surfaces disclosed numerous petechiae throughout the parenchyma. Both cortices and medullas of cases 6 and 7 were considerably paler than normal and the usual demarcations were definitely obscured. In addition gray foci measuring 1 mm. in diameter were found throughout the kidneys of case 6 and the pelves and ureters of cases 1 and 7 were intensely congested and contained recently clotted blood.

Brain. The brain was examined in cases 1, 2, and 4. In case 2 it weighed 1,260 gm. Externally it was normal but within its substance it contained two recent hemorrhages each measuring 0.5 cm. across and situated, respectively, in the white substance of the right frontal and parietal lobes. Each brain in the other 2 cases weighed 1,500 gm. In case 1 it was symmetrically enlarged, whereas in case 4 there was some bulging of the left parietal lobe. Extravasation of blood in the subarachnoid space was slight in case 1 and marked in case 4. In each the gyri were broad and flat and the sulci were obliterated. Coronal sections disclosed the ventricular system in each brain to be filled with fluid and clotted blood. In case 1 there were six separate hemorrhages irregularly distributed throughout the cerebral hemispheres, but there were none in the brain stem and cerebellum. In case 4 most of the left parietal and occipital lobes were destroyed by a massive recent hemorrhage measuring 7.5 by 6 cm. In addition there were scattered small hemorrhages into what remained of the left parietal lobe and into the left temporal lobe. The brain stem and cerebellum were normal.

Other Viscera. There were numerous petechiae in the pericardium of cases 1, 2, and 4, in the endocardium of case 4, and in the pleura of cases 1, 2, and 7. The lungs of all cases showed marked congestion

and edema. Hemorrhages measuring as much as 6 cm. in diameter were irregularly distributed throughout the lungs in cases 1, 3, 4, and 6, and raised, gray, indefinitely circumscribed foci measuring up to 1 cm. across were present in those of cases 1, 3, 6, and 8. The remaining thoracic and abdominal organs showed no contributory changes.

Microscopic Pathologic Observations

Sections of all organs were stained with hematoxylin and eosin. In addition, triplicate sections of the spleen, lymph nodes, and bone marrow of all cases and of the liver of some cases were stained with hematoxylin and eosin, Giemsa's stain, and Foot's reticulum stain. Stains for oxidase granules in histologic sections were carried out on tissue from some of the cases using, as control material, tissue from a case of myeloid leukemia stored for approximately the same time. None showed peroxidase granules, probably due to the fact that all material was too old.

The Monocyte in Histologic Sections

The monocytic cells within vessels were more uniform than were those infiltrating the tissues. They were round or oval, and one and one-half to three times the diameter of erythrocytes (Figs. 1 and 2). The cell borders were usually distinct and sharp but occasionally they were ill defined and fuzzy. The cytoplasm occupied one-eighth to three-quarters of the cell volume and with hematoxylin and eosin stained deep pink, whereas it was stained light blue by the Giemsa method. Sometimes the cytoplasm was proportionately more abundant and contained phagocytosed nuclear material, brown pigment, and erythrocytes (Fig. 3). The nuclei were ordinarily relatively large and were round, oval, or irregular. The irregular nuclei often had a single indentation giving a typical horseshoe-shaped appearance, but also they frequently contained two or more deep indentations which gave the appearance of definite lobulations. Occasionally the nuclear material was irregularly twisted upon itself, necessitating fine focusing to distinguish the convolutions. The nuclear margins were always distinct but the nucleoplasm generally stained very lightly and contained chromatin grouped in small, irregular aggregations, sometimes connected by fine, almost imperceptible threads. Nucleoli were not seen in any of the intravascular monocytic cells.

The monocytic cells within the alveolar spaces of the lungs possessed essentially the same characteristics as those within vessels except that they were slightly larger, somewhat more irregular, showed more phagocytosis, and many mitotic figures (Fig. 4). The nuclei were per-

haps more darkly stained and generally more irregular than were those in the circulating blood. Nucleoli were not seen.

In tissues the cells showed considerably more pleomorphism. When they were more mature and less tightly packed they closely resembled those in the pulmonary alveolar exudate and circulating blood. When, however, they were less mature and more crowded, all gradations were seen from the round or oval cells just described to large spindle-shaped, polyhedral or irregular forms which were indistinguishable from regular reticulum cells (Fig. 5). Often long, thin, fibrillary processes projected from the angles of the more irregular cells and were directly continuous with the supporting reticulum (Figs. 6 and 7). At other times the sinus endothelial cells, particularly in the bone marrow, were swollen and showed varied degrees of detachment both intravascularly and extravascularly until the cytoplasm was completely separated (Fig. 8). The nuclei of all cells mentioned were similar in staining qualities but varied in size and external configuration. In the reticulum cells and in the more immature spindle and irregular monocytic cells they were considerably larger than in the round and oval circulating monocytes. Often they were of irregular shape, assuming triangular and rectangular configurations. They showed few twists and indentations, and rarely assumed the characteristic horseshoe shape. Occasionally there appeared to be two or three separate nuclei piled up in a single cell to produce what closely resembled Sternberg-Reed giant cells. One or two nucleoli were seen in some of the more immature cells when stained by the Giemsa method. They could not be definitely identified in the routine sections stained with hematoxylin and eosin. Mitotic figures were numerous among the immature cells but were less frequent or entirely absent among the more mature monocytes.

Skin. Histologic sections of the skin were available from cases 1, 2, 4, 6, and 7. The epidermis was normal. The immediately subjacent dermis was edematous but the remainder was dense and collagenous. Hemorrhagic extravasation was present in all cases, reaching a maximum degree in case 4. Usually, small collections of erythrocytes were insinuated between the collagen fibers throughout the dermis. Sometimes they were adjacent to capillaries but at other times they appeared to be independent of vessels. In case 4 there were, in addition, massive hemorrhages into the subcutaneous tissue. Infiltrations of leukocytes were also present in all cases. In the superficial portions of the dermis, particularly in the edematous zone, they usually surrounded small capillaries, but in the deeper layers this relationship was less apparent. The most severe infiltrates were grouped around hair shafts and sebaceous and sweat glands (Fig. 9). Whereas in some instances

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(case 1) the infiltrating leukocytes were all of the monocytic variety, in others (case 2) there were present also a few myeloid cells. In all cases erythrocytic extravasation appeared to be entirely independent of the leukocytic foci.

Gingivae. Sections of the gums were available from case 1. The mucosa was intact and, except for some vacuolization of the prickle cell layer, was normal. The immediately subjacent submucosa was edematous but contained only a few empty capillaries and was sparsely infiltrated with round or oval monocytes (Fig. 10). In the deeper tissues, however, the monocytes were so densely packed that they obliterated all the normal structures with the exception of a few strands of striated muscle. In these areas the monocytic cells were polygonal and quite irregular, showed scattered mitotic figures, and were not particularly disposed about blood vessels. Throughout the sections there were only a few widely separated and insignificant foci of extravasated erythrocytes.

Spleen. Histologically, the spleens of cases 1, 3, 5, 6, and 7 were essentially the same. In each the capsule was thin and the trabeculae were decreased relatively in number and size. These spleens contained varied numbers of monocytes and erythrocytes. Normal follicles were practically nonexistent (Fig. 11). In some the arterioles were quite prominent and were surrounded not by lymphocytes but by cuffs of large, irregular, immature monocytes that were often in a state of mitosis (Fig. 12). Sprinkled among these cells were a few polymorphonuclear leukocytes and lymphocytes. The periphery of each periarteriolar collection was sharply separated from a pulp infiltrated with somewhat more regular and smaller monocytic cells. The infiltration of the latter was so dense that the underlying sinusoidal spaces could not be recognized. There was, however, no apparent hyperplasia of the reticulum of the pulp and erythrocytes were not plentiful. Eosinophils were present in the spleens of cases 5 and 7. The former, in addition, disclosed several areas containing relatively large single or multinucleated giant cells that were indistinguishable from Sternberg-Reed cells (Fig. 13). Sections from the spleen of case 2 differed from the foregoing in that they were less homogeneous. The normal periarteriolar collections of lymphocytes were replaced with solid cuffs of hyperplastic reticulum, between the meshes of which were many monocytes, fewer phagocytes laden with golden brown pigment, polymorphonuclear leukocytes, and lymphocytes (Figs. 14 and 15). The pulp was less solid than in the previously described cases and in many areas there was a definite increase of fine reticulum with which the monocytes were intimately bound and often connected by fine processes. The

spleen of case 4 was even less homogeneous. Normal follicles were present in some areas while in other areas both the follicles and the pulp were completely replaced with a solid infiltration of monocytes with fewer myeloid and erythroid cells. Between these solid areas the reticulum showed much hyperplasia and widely patent sinusoids (Fig. 16). The endothelium of the latter showed patchy or continuous hyperplasia and an extrusion of some of the lining cells into the lumen to form free monocytes. When less densely packed, the monocytic cells in the intersinusoidal spaces were seen to be intimately connected with the underlying reticulum (Fig. 17). In some areas the presence of mononuclear giant cells and eosinophils presented a picture not unlike that of Hodgkin's disease. The spleen of case 8 was so crowded with erythrocytes that the underlying architecture was completely obliterated. Beneath the capsule, however, there were collections of large irregular monocytes and fewer lymphocytes, plasma cells, and polymorphonuclear leukocytes which, but for the absence of eosinophils, resembled Hodgkin's disease.

Liver. The distribution of the leukemic infiltrates in the liver was not uniform. In cases 1, 2, 5, and 6 the portal areas were decidedly more involved than were the lobules, but both participated in the process (Fig. 18). In the former there were varied degrees of reticulum hyperplasia. This reached a maximum degree in case 1 where the cells around the vessels often assumed a more or less whorled formation. Throughout these areas mitotic figures were quite common and there were all transitions from large, polyhedral cells with abundant cytoplasm and long, fibrillary processes to the relatively mature, round or oval monocytes. Other leukocytic cells were almost entirely absent. Perisinusoidal edema throughout the lobules rendered the sinusoids particularly conspicuous. Some lining endothelial cells showed various degrees of swelling until they were completely detached to form intrasinusoidal phagocytes (Figs. 19 and 20). Both Kupffer's cells and the intrasinusoidal phagocytes contained ingested brown pigment and nuclear fragments. Other swollen endothelial cells showed no phagocytosed material and appeared to be gradually extruded into the lumina as monocytes in varying numbers and in various stages of maturity. Mitotic figures were present only occasionally. The nuclei of the swollen endothelial cells were identical with those of both the intrasinusoidal phagocytes and the monocytes. The liver cells were somewhat atrophic but otherwise were well preserved except in case 2 where they showed severe central degeneration. Lipoidosis was patchy, and mitotic figures were present in some of the hepatic cells of case 1. Infiltrations of leukemic cells in the livers of cases 4 and 7 were practi-

cally confined to the liver cords with almost no involvement of the portal canals (Fig. 21). In case 7, however, only about 20 per cent of the cells were monocytic while the rest were of the myeloid series. The livers of cases 3 and 8 were similar in that the distribution of leukemic cells in the portal areas and the sinusoids was about equal. In case 3 it was less extensive than in case 8. In the former, however, most of the cells were monocytes, whereas in the latter only about 10 per cent were monocytic and the rest were myelocytic. Convincing transitions from Kupffer's cells to free monocytes were demonstrable in the liver of case 3 but not in the livers of cases 4, 7, and 8.

Bone Marrow. The bone marrow of the sternum was consistently more involved than was that of the ribs, vertebrae, and femora. It was extremely dense in cases 1 and 7 (Figs. 22 and 23), and least of all in case 8. The monocytic cells were most immature in cases 1 and 7 where they constituted from 85 to 100 per cent of the marrow cells. In case 1 all gradations could be traced from ordinary reticulum cells and sinus endothelial cells to mature round or oval monocytes. Elongated and irregular polygonal cells with long processes attached both to sinus endothelium and underlying reticulum were particularly conspicuous (Figs. 5 and 6). In case 7, although the cells were irregular, fibrillary processes were absent. In both cases fat cells were entirely crowded out, but between the leukemic cells there were scattered remnants of myeloid, erythroid, and megakaryocytoid cells. In cases 2, 3, 4, 5, and 6 the marrow, although diffusely involved, was less densely packed than it was in the aforementioned cases, and there were occasional scattered fat cells. The number of monocytic cells, as compared with other marrow elements, varied from 60 to 100 per cent. In all of these cases, however, the leukemic cells were more mature and were thus usually round or oval as compared with the more prevalent immature and irregular cells seen in the 2 former cases; hence fibrillary processes and transitions to reticulum cells were not apparent.

The marrow of case 8 was in a separate category. The right femur, vertebrae, ribs, and sternum were examined histologically and were all essentially similar. The general architecture of the marrow was little disturbed, showing an abundance of fat and other marrow cells. Throughout the sections, however, there were conspicuous reticulum cells and huge monocytes (Fig. 24). Usually the leukemic cells were sparsely scattered and represented not more than 10 per cent of all cells, but sometimes they were collected into small foci in which they constituted about 80 per cent of the marrow cells. Many of the monocytes were irregularly polygonal with pointed angles from which fibrillary processes emerged to join the underlying reticulum. Their cyto-

plasm was relatively scanty and even in sections stained with hematoxylin and eosin was light blue. The nuclei were lightly stained, round or oval, and similar to those of other monocytic cells except that they were considerably larger. Mitotic figures were abundant. Although definite connections of these cells with the sinus endothelium could not be demonstrated, the nuclei of swollen endothelial cells were very similar to those of the monocytic cells. In none of the cases was the cortical bone penetrated or the periosteum stimulated to produce new bone.

Lymph Nodes and Thymus. Although the lymph nodes of all cases were involved in the leukemic process, the degree of infiltration varied considerably. Those of case 3 were least affected. Follicular architecture was fairly well preserved but scattered throughout the nodes were round or oval monocytic cells comprising about 25 per cent of all free cells. There were present, also, numerous phagocytes containing golden brown pigment and only very few eosinophils. The nodes of case 4 were slightly more involved. The follicles were still recognizable and consisted of 100 per cent lymphocytic cells. Between these, however, there were broad, branching bands of densely packed reticulum cells which showed transformation to both monocytes and large mononuclear phagocytes (Fig. 25). The nodes of cases 2, 5, 6, and 7 were essentially similar. Except for a few scattered atrophic follicles, the normal architecture was completely replaced by a diffuse infiltration with monocytic cells (Fig. 26). They were round or oval and showed no processes or attachments either to the reticulum or sinus endothelium. The reticulum was increased only focally around the thicker vessels in the medulla of some of the nodes (Fig. 27). The capsules were uniformly infiltrated with monocytic cells. In cases 2 and 7, however, the presence in the less densely infiltrated areas of a few plasma cells, polymorphonuclear leukocytes, eosinophils, and binucleate or multinucleate giant cells produced an appearance very similar to that of Hodgkin's disease (Fig. 28). The lymph nodes of case 1 were perhaps most severely involved. The cortices were completely replaced with closely packed monocytic cells which were nevertheless round or oval. Mitotic figures were abundant. The medullas, particularly around the thicker blood vessels, showed a severe degree of reticulum hyperplasia with numerous polygonal and elongated cells that often assumed a sarcomatous appearance. From these there were transitions to typical monocytes, but in many instances it was impossible to label the cells as one or the other with any degree of assurance. Although generally the medullas were sharply separated from the cortices by a fine connective tissue capsule, in several areas the latter was interrupted and the cells in the two zones were freely intermingled (Fig.

29). The nodes of case 8 were replaced with less densely packed cells than were those of the other cases. Huge single and multinucleated giant cells indistinguishable from Sternberg-Reed cells were very conspicuous everywhere. Smaller monocytes, although present, were less prevalent (Fig. 30). The reticulum was generally increased and often was intimately connected with the irregular monocytic cells. The presence of phagocytes, few plasma cells, myeloid cells, and lymphocytes produced a picture somewhat resembling that of a "sarcomatous" type of Hodgkin's disease. Sections of the thymus from cases 1 and 5 were examined and in each they were similar to the sections of lymph nodes from the respective cases (Fig. 31).

Intestines. Involvement of the lymphoid apparatus of the intestine paralleled that of the lymph nodes. In some of the cases there was little or no infiltration with leukemic cells. In others, as in case 5, the follicles were normal but the pulp disclosed a definite increase in monocytes. Except for ulceration of the overlying mucosa, there were no other changes in the bowel wall. Peyer's patches in case 1 showed the most severe involvement. The lymph follicles were prominent but were not normal. The germinal centers of all of them were enlarged and completely replaced with large, irregular reticulum cells and monocytes (Fig. 32). At the periphery they were surrounded by a thin, compressed rim of lymphocytes, beyond which the pulp was again entirely replaced with reticulum cells and monocytes. The covering mucosa, as in case 5, was ulcerated. In case 7 the mucosa of the small intestine was intact but congested. The submucosa was thickened and edematous in patchy areas and was sparsely infiltrated with monocytes. Edema and fibrosis of the submucosa reached its maximum degree in the rectum of case 6 where it measured 1 cm. thick. Both the submucosa and serosa were heavily infiltrated with monocytes but the muscle contained only scattered cells.

Kidneys. The kidneys of cases 2, 4, 5, 6, and 7 were involved in the leukemic process. The infiltrates of monocytic cells were primarily interstitial and in the cortex, and only when very severe was the medulla involved. In cases 2 and 4 the foci were small. They were larger in cases 5 and 6 (Fig. 33) and in case 7 there was a diffuse infiltration of both the cortex and medulla. When the foci were small, the renal architecture was not disturbed, but as they increased in size there was first atrophy and later complete replacement of all the normal structures.

Other Organs. There were submucosal hemorrhages and surrounding monocytic infiltrations in the ureters of case 7 and the bladder of case 1, and a diffuse interstitial infiltration with leukemic cells in the

testes of cases 1 and 7. In the heart there were subendocardial foci of monocytes in cases 4, 6, and 7, pericardial foci in cases 1 and 7, and myocardial foci in case 7. Congestion and edema were present in the lungs of all cases and hemorrhages in those of cases 1, 4, 5, and 6. Exudate of monocytic cells, which appeared somewhat larger than those in the circulating blood, was present in the septa and alveolar spaces in cases 1, 2, 3, 5, 6, and 7, in reality producing a monocytic pneumonia (Fig. 4). In addition to the monocytes, some of the exudate contained scattered fibrin threads and a few large phagocytic cells exhibiting ingested débris. In the adrenals, leukemic infiltrations of monocytic cells were present in the inner zones of the cortices, in the medullas, or in the periadrenal connective tissue of cases 1, 2, 6, 7, and 8. There was a diffuse interstitial infiltration of monocytes in the pancreas of case 7. The remaining thoracic and abdominal organs showed no contributory changes. Sections of the brain in each of the cases in which it was examined (1, 2, and 4) disclosed monocytic cells filling the vessels and extravasated beneath the meninges and into the areas of hemorrhage. Occasionally, there were scattered foci within the white matter, apparently independent of blood vessels. About some of the arterioles in case 2 there was an adventitial proliferation of reticulum cells and what appeared to be transformation of these cells into monocytes (Fig. 34). The brain substance around the hemorrhages was edematous and vacuolated but showed no attempt at repair.

Comment

Participation of the reticulo-endothelial system in monocytic leukemia has been variously reported. Some authors have not commented upon it, others have noted an increase in reticulum cells,[2,3,7-13] and still others have described an absence of their proliferation.[11,14,15] In some of our cases there was a definite hyperplasia of the reticulo-endothelial system with a traceable transformation of the cells into monocytes, while in other cases such changes were not demonstrable. The explanation for this disparity is apparently two-fold. First, the age of the patient appears to be important. It seems as though the younger the individual (cases 1 and 2) the more severe and profuse will be the response and, conversely, the older the individual (case 8) the less extensive will be the proliferative process. Secondly, of equal importance and coupled with the age of the patient appears to be the amount and duration of the stimulus. When the disease is fulminating, and presumably when the stimulus is more potent, there will be such a rapid proliferation of reticulum cells that their maturation into mono-

cytes cannot keep pace, resulting in an accumulation in those locations where they are usually most abundant. When, however, the disease is more protracted, and presumably when the stimulus is less, there will be a slower degree of proliferation of reticulum cells resulting in their more subtle and complete transformation into monocytes. Under these circumstances there is neither an evident accumulation of reticulum cells nor is there a traceable differentiation of these cells into monocytes.

Although Kupffer's cells are a part of the reticulo-endothelial system, a review of the literature discloses that their transformation into monocytes has never been satisfactorily demonstrated. We believe that in some of our cases such a transition was unquestionably encountered. A careful study of many sections of the livers disclosed a swelling of the nuclei and cytoplasm of the Kupffer cells with a gradual bulging into the sinusoidal lumina and eventually a complete detachment to form free intrasinusoidal cells. While some of these became ordinary phagocytes, others became rounded off to form circulating monocytic cells. As would be expected, the nuclei of the swollen endothelial cells, phagocytes, and monocytes were identical. Mitotic figures, however, were only rarely seen in the Kupffer cells.

Several authors have noted a striking histopathologic similarity between subacute and chronic monocytic leukemia and Hodgkin's disease.[4,8,16,17] While we, too, encountered such a resemblance in the spleens of cases 4, 5, and 8, and in the lymph nodes of cases 2, 7, and 8, it is to be noted that in all of these the disease was fulminating, and that in case 6, in which the disease was more protracted, there was no such similarity. It is possible, therefore, that the various types of leukocytic cells producing a picture of Hodgkin's disease are found when the stimulator or stimulators first hit the tissues and when all cells proliferate. Later, when the action of the specific stimulator becomes stabilized, only one type of cell is produced—the monocyte—and this floods and replaces the previous heterogeneous mixture of leukocytic cells with a homogeneous one.

Finally, it is noteworthy that hemorrhages and leukemic infiltrations were present in each brain examined. In one of these (case 2) the hemorrhage was small and inconsequential, but in the remaining two (cases 1 and 4) it undoubtedly contributed to, or was the immediate cause of, death. In a recent review of the brain in leukemias, Leidler and Russell [18] studied a total of 67 cases, of which only 3 were of the monocytic variety. In 62 there were leukemic infiltrations and/or hemorrhages, and the latter were of sufficient degree to be the immediate or contributory cause of death in 27 cases or 40 per cent.

Summary and Conclusions

By a detailed study of the histopathologic changes in 8 cases of monocytic leukemia, it was shown that reticulo-endothelial cells are unquestionably the precursors of monocytes. Their demonstrable proliferation and transitions to the freely circulating cell appear to depend upon the age of the patient and the degree of stimulation. A fulminating course in a young person will produce extensive reticulum hyperplasia because the maturation of the cells cannot keep pace with proliferation. In an older person a similarly acute course will produce less hyperplasia because the tissues are less adaptable to proliferation. In the latter, as well as in protracted cases, there is a more subtle and complete transformation of the reticulo-endothelial cells into monocytes and the transitions are more difficult to demonstrate.

Like all reticulo-endothelial cells, the Kupffer cells of the liver were seen to participate in the formation of monocytes in some of our cases. All transitions from initial swelling to complete extrusion into the sinusoidal lumina were encountered.

In our cases a heterogeneous accumulation of leukocytic elements, producing a picture resembling Hodgkin's disease, was found in the acute cases. This is in contrast to the reports in the literature in which this feature is described as occurring in the subacute and chronic forms of the disease.

The brain was examined in 3 cases. In each of these there were leukemic infiltrates and hemorrhages. The latter were of sufficient degree to be a contributory or immediate cause of death in 2 cases.

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DESCRIPTION OF PLATES

PLATE 15

FIG. 1. Circulating monocytes in a pulmonary blood vessel of case 7, with many spherical cells with round or indented nuclei and a moderate amount of cytoplasm. Hematoxylin and eosin stain. × 400.

FIG. 2. Circulating monocytes of case 7 at a higher magnification, showing indented, round, or irregular nuclei with lightly stained chromatin. Hematoxylin and eosin stain. × 900.

FIG. 3. From a vein in the liver of case 1, showing closely packed monocytes and large intravascular macrophages containing ingested material. Hematoxylin and eosin stain. × 400.

FIG. 4. Monocytic exudate into the lung of case 1. The cells are slightly larger than those within vessels. They are round, have distinct borders and nuclei, and some are in mitosis. A few phagocytic cells are present also. Hematoxylin and eosin stain. × 400.

FIG. 5. From the thymus from case 1, showing an intimate mixture of reticulum cells and monocytes in various stages of maturity. Hematoxylin and eosin stain. × 400.

FIG. 6. Another area from the thymus from case 1, showing monocytes with long tail-like processes attached to the related reticulum. Hematoxylin and eosin stain. × 900.

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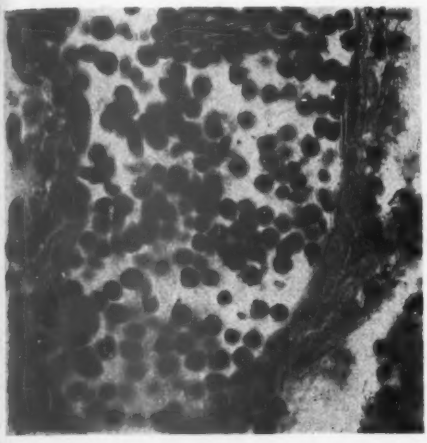

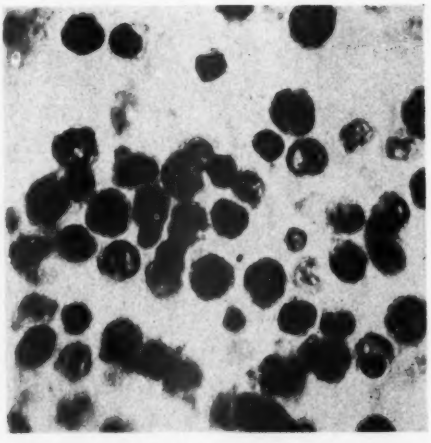

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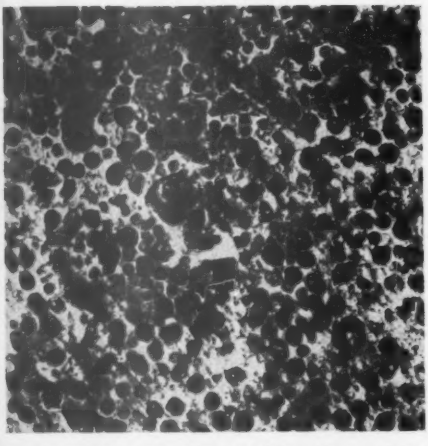

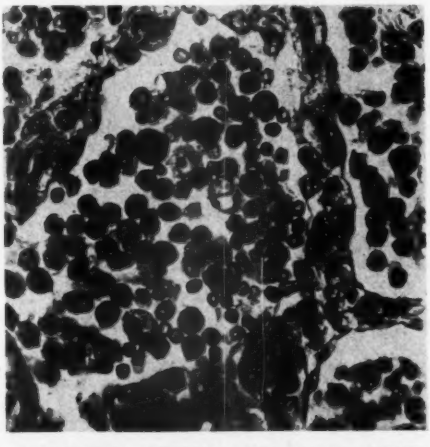

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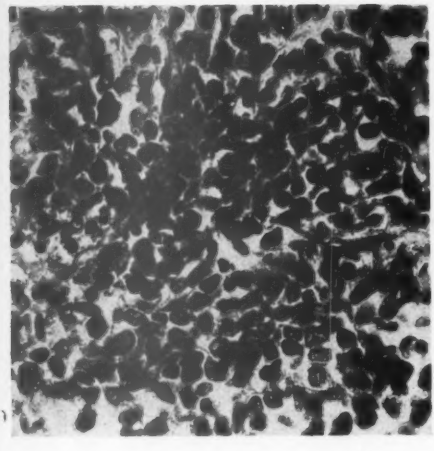

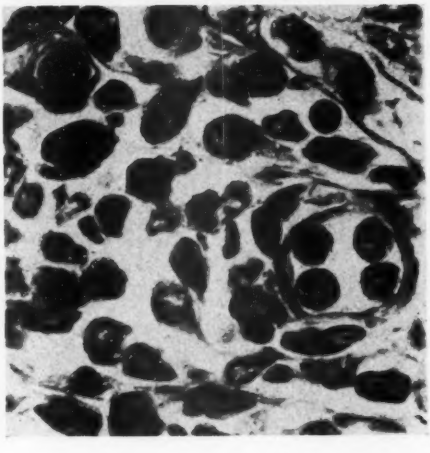

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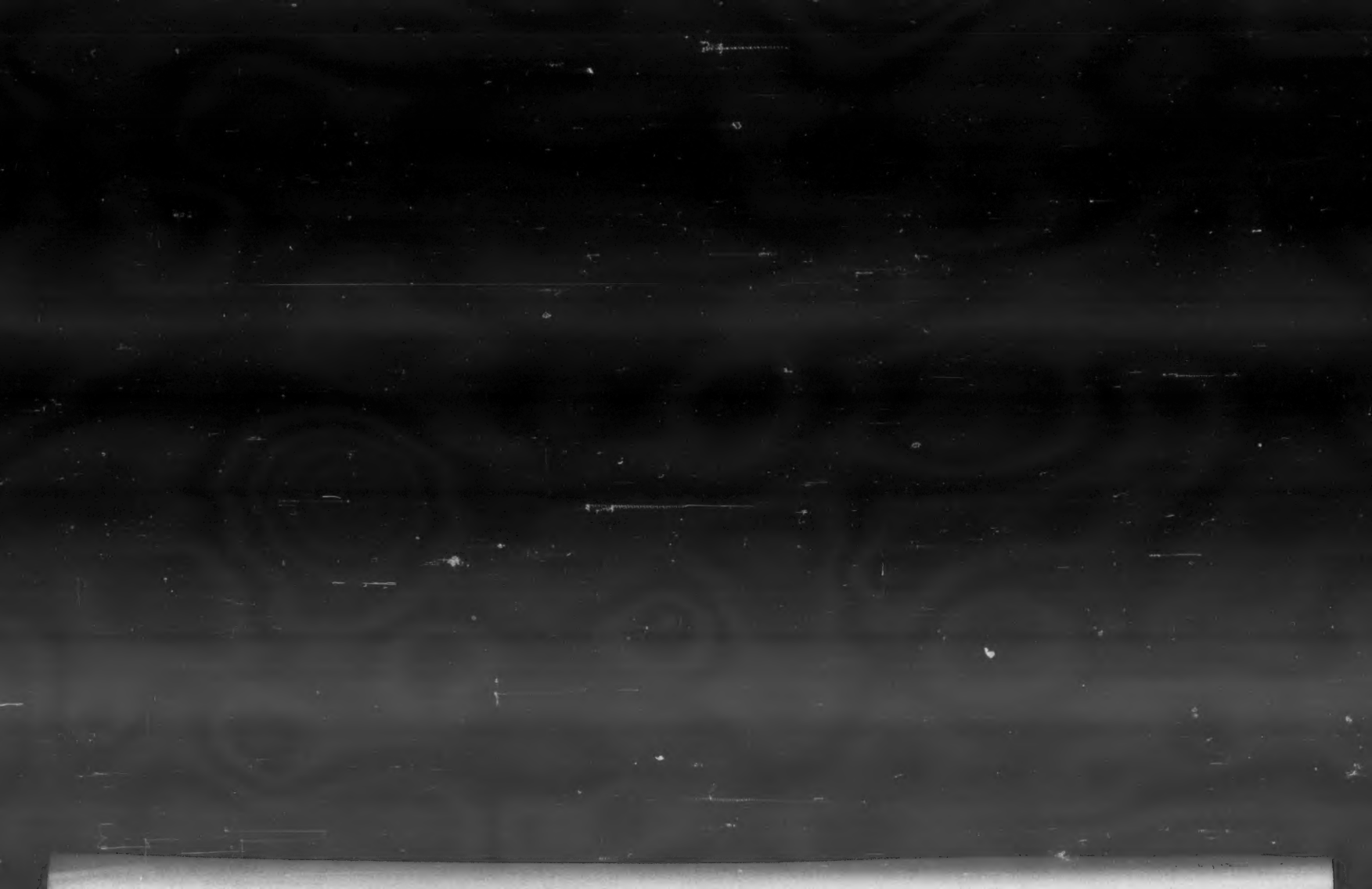
Plate 16

Fig. 7. The vertebral bone marrow from case 1, showing some monocytes intimately connected with the underlying reticulum. Hematoxylin and eosin stain. × 400.

Fig. 8. Another area from the vertebral bone marrow of case 1 shows bulging of the sinus endothelium and extrusion to form monocytes. Hematoxylin and eosin stain. × 400.

Fig. 9. Section of the skin from case 7, showing monocytic infiltration about a hair shaft. Hematoxylin and eosin stain. × 50.

Fig. 10. Section of the gingiva from case 1, showing intact epithelium and edematous submucosa infiltrated with monocytes. Hematoxylin and eosin stain. × 50.



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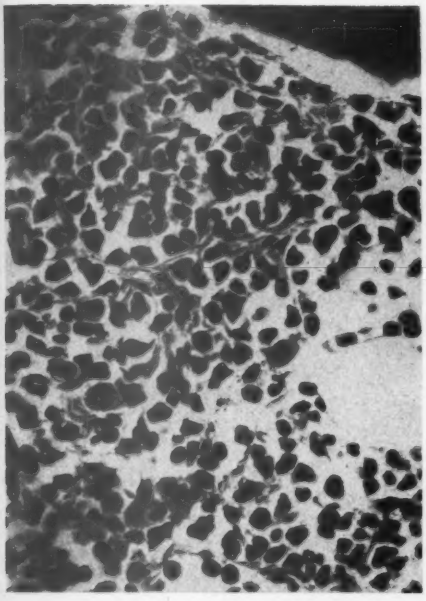

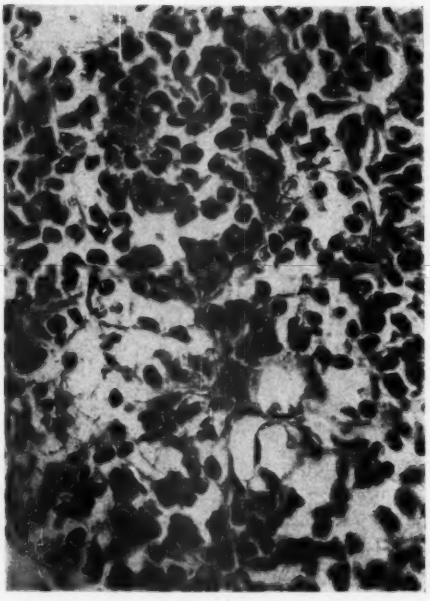

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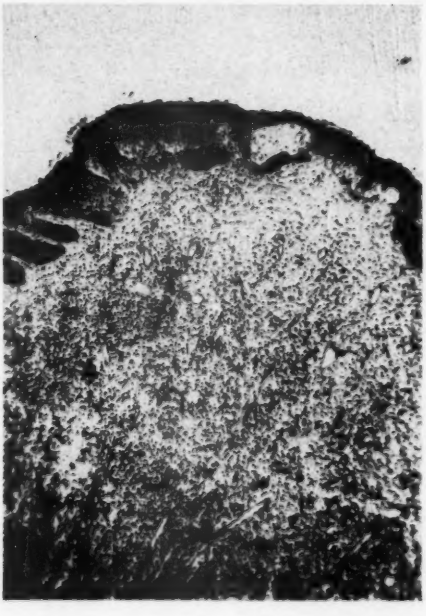

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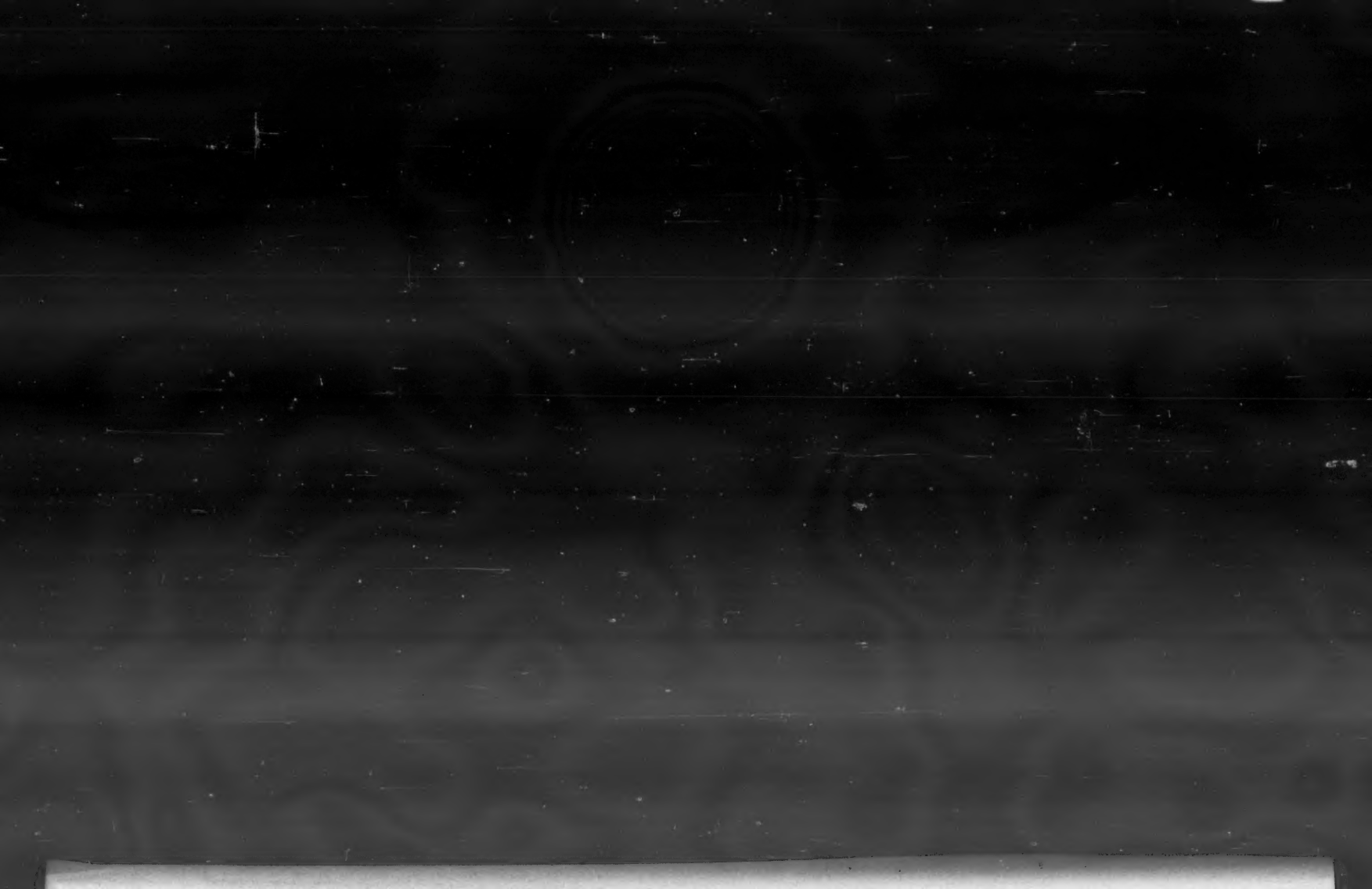
PLATE 17

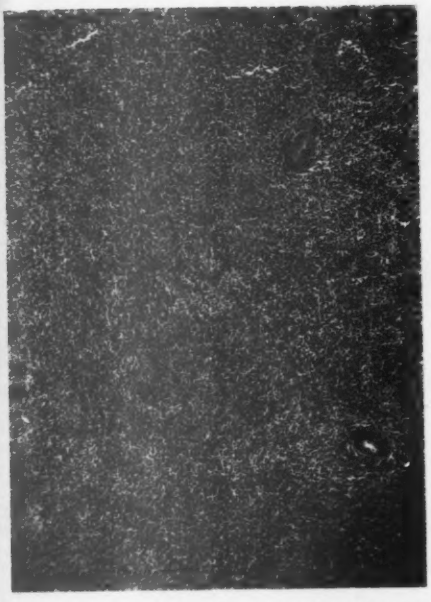
FIG. 11. The spleen from case 6 shows complete effacement of the normal architecture. Hematoxylin and eosin stain. × 50.

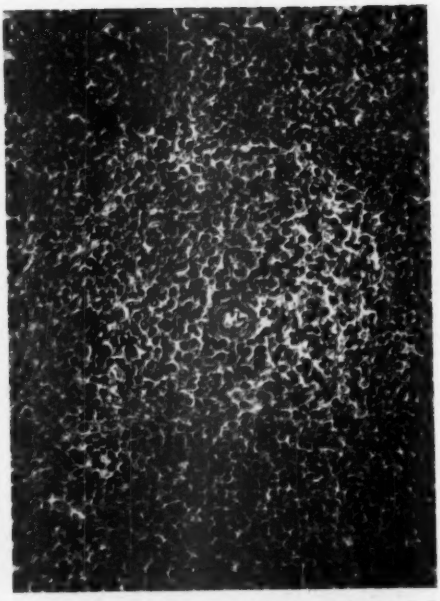
FIG. 12. Section of the spleen from case 1, showing an arteriole surrounded by immature monocytes sharply demarcated from the pulp. Some of the cells are in mitosis. Scattered sparsely among the monocytic cells there are a few polymorphonuclear leukocytes and lymphocytes. Hematoxylin and eosin stain. × 200.

FIG. 13. The spleen from case 5 presents a picture resembling Hodgkin's disease. Mononuclear and multinuclear giant cells of the Sternberg-Reed type, eosinophils, and other leukocytic cells are present. Giemsa's stain. × 200.

FIG. 14. Section of the spleen from case 2, showing marked periarteriolar reticulum cell hyperplasia. Hematoxylin and eosin stain. × 50.







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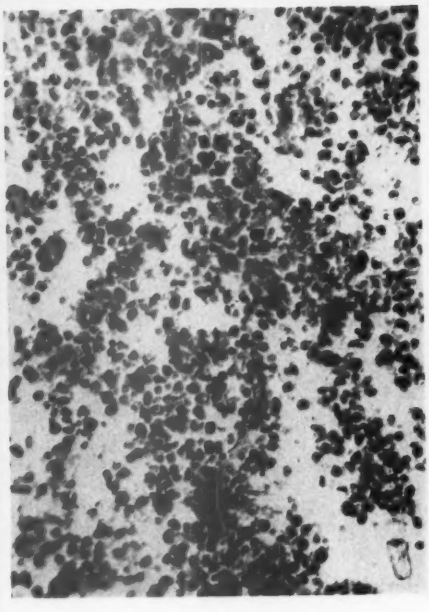

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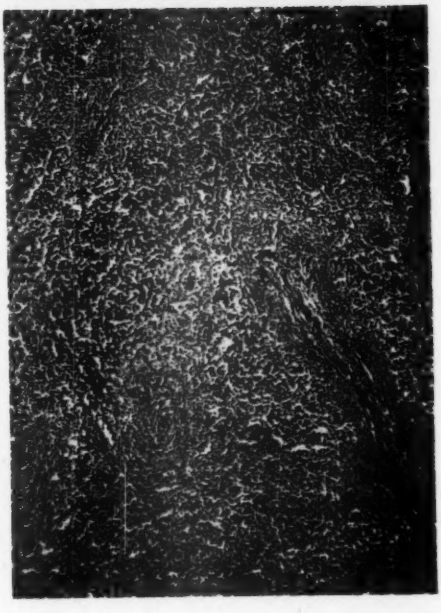

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PLATE 18

FIG. 15. A marked increase of reticulum around an arteriole of the spleen from case 2 is shown by Foot's reticulum stain. × 200.

FIG. 16. Section of the spleen from case 4, showing a widely patent sinusoid lined with swollen endothelial cells. Some of these cells have been extruded into the lumen as monocytes. Hematoxylin and eosin stain. × 400.

FIG. 17. Another section of the spleen from case 4, showing an intimate relation between the reticulum and monocytes. Hematoxylin and eosin stain. × 400.

FIG. 18. The liver from case 2 shows a heavy infiltration of the portal areas with leukemic cells. Hematoxylin and eosin stain. × 50.

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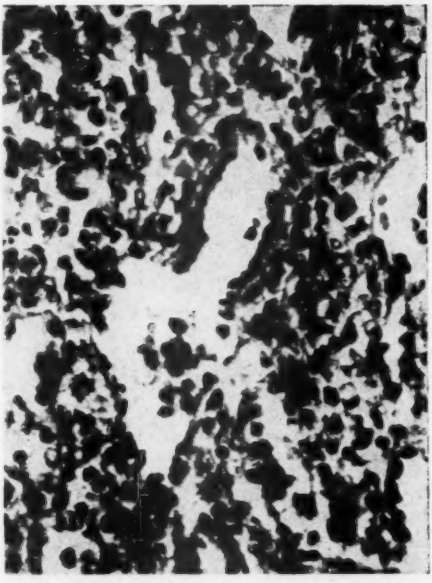

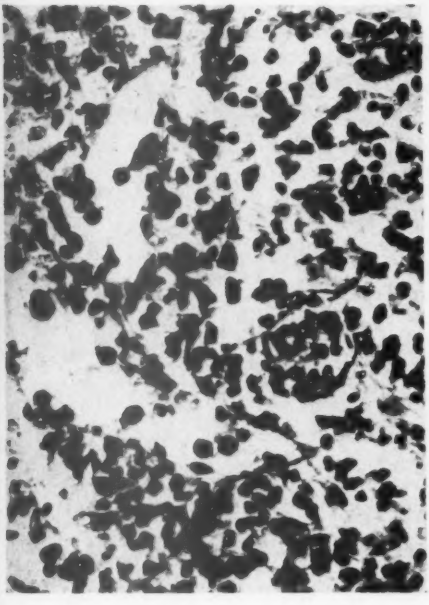

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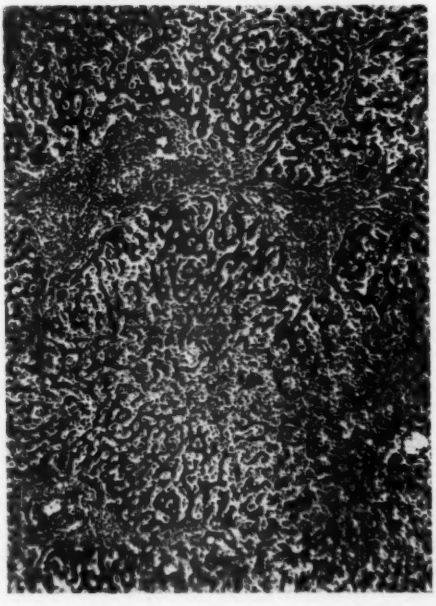

PLATE 19

FIG. 19. Section of the liver from case 1, showing swelling of the sinus endothelial cells with extrusion of some of the cells into the lumen. One of the extruded cells in the center of the photomicrograph is in mitosis. Hematoxylin and eosin stain. × 400.

FIG. 20. Another area of the liver from case 1 to show Kupffer's cells in various stages of swelling and detachment into the lumina. Hematoxylin and eosin stain. × 400.

FIG. 21. Section of the liver from case 7, showing leukemic infiltration between the hepatic cords and its absence in the portal areas. Hematoxylin and eosin stain. × 50.

FIG. 22. The vertebral bone marrow from case 1 shows an almost complete replacement of the normal cells by monocytes, with aggregation of the latter around a blood vessel. Hematoxylin and eosin stain. × 50.

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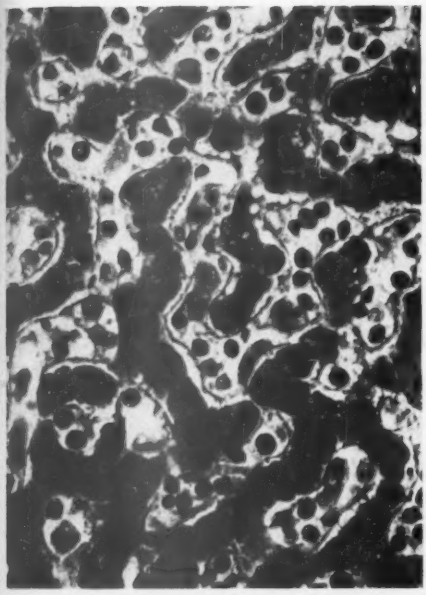

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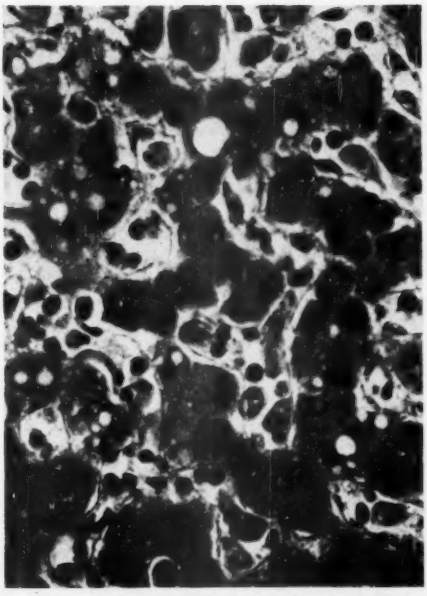

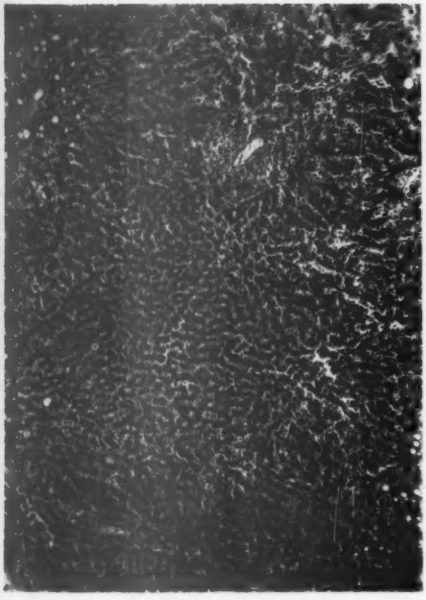

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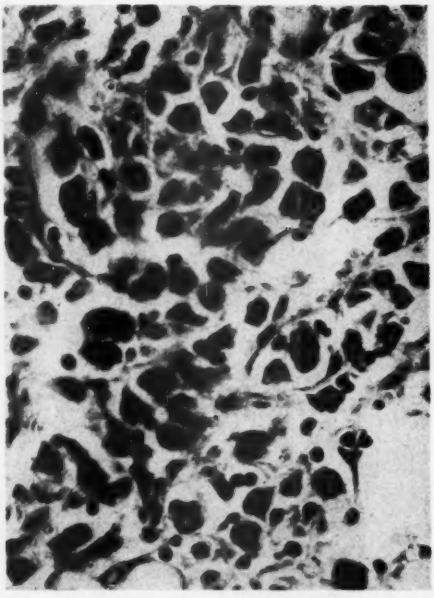
PLATE 20

FIG. 23. Section of sternal bone marrow from case 7, showing a complete replacement of the normal cells with monocytes. Hematoxylin and eosin stain. × 50.

FIG. 24. The sternal marrow from case 8 shows huge monocytes, some of which have thin processes attached to the intermingled reticulum. Hematoxylin and eosin stain. × 400.

FIG. 25. Section of a lymph node from case 4, showing marked hyperplasia of the sinus reticulum with crowding of the lymphoid elements. Hematoxylin and eosin stain. × 75.

FIG. 26. A lymph node from case 7 shows complete replacement of the normal architecture by monocytes and an infiltration of the capsule by similar cells. Hematoxylin and eosin stain. × 50.



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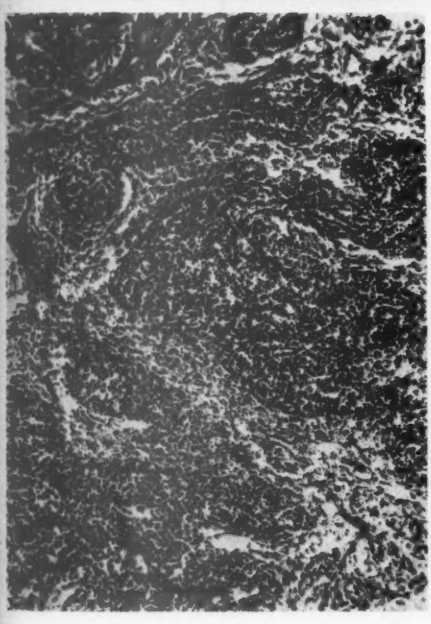

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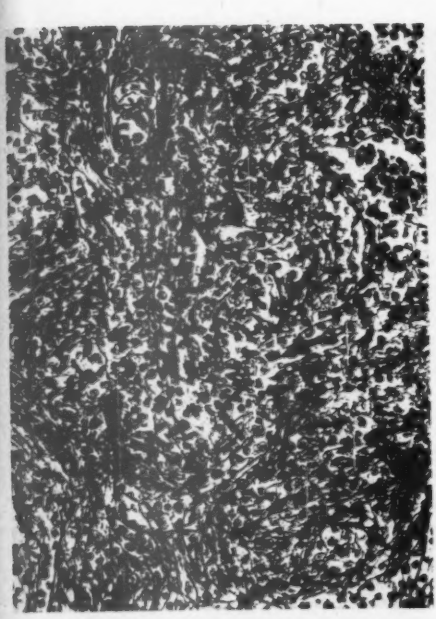
PLATE 21

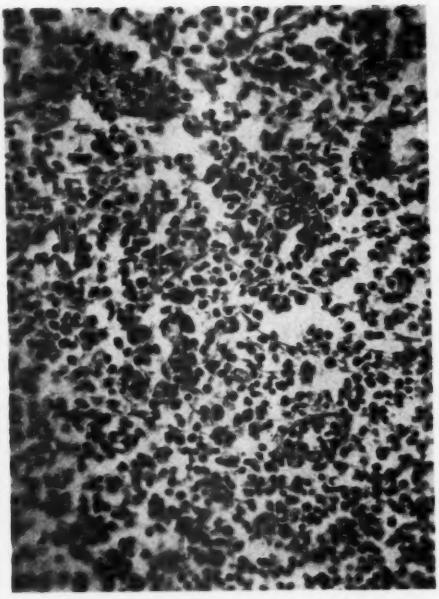
FIG. 27. Section of a lymph node from case 7, showing an area of reticulum hyperplasia. Foot's reticulum stain. × 200.

FIG. 28. Section of a lymph node from case 2, showing a picture resembling Hodgkin's disease. Large cells of the Sternberg-Reed type, eosinophils, lymphocytes, and a few polymorphonuclear leukocytes are present. Giemsa's stain. × 200.

FIG. 29. This area from a lymph node from case 1 shows at the lower right marked reticulum cell hyperplasia of the medulla with some maturation into monocytes. At one point the thin capsule is broken and there appears to be an outpouring of somewhat more mature monocytes into the cortex. Hematoxylin and eosin stain. × 200.

FIG. 30. Section of a lymph node from case 8, showing a picture somewhat similar to a "sarcomatous" type of Hodgkin's disease. The giant cells are larger, have more cytoplasm and more irregular borders than do those illustrated in Figures 13 and 28. Phagocytes, plasma cells, lymphocytes, and polymorphonuclear leukocytes are also present. Throughout this section there is a slight increase of the associated reticulum. Hematoxylin and eosin stain. × 200.





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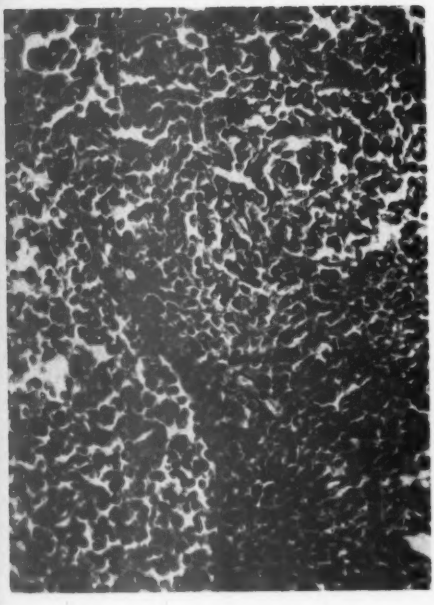

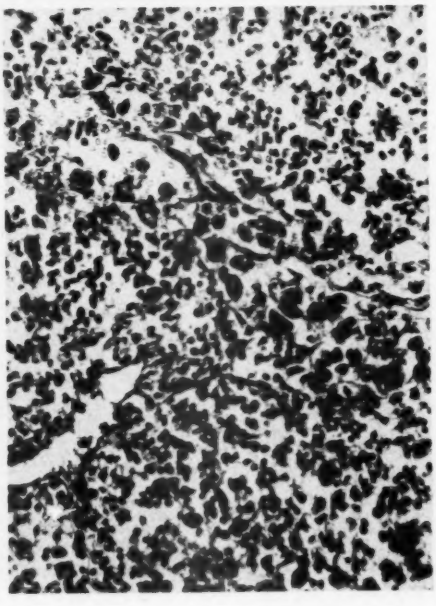

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PLATE 22

FIG. 31. Section of the thymus from case 1, showing a diffuse increase of reticulum in areas with reticulum cell hyperplasia, but a paucity of reticulum in areas containing mature monocytes (upper left corner). Foot's reticulum stain. × 200.

FIG. 32. Section of a germinal center in the small intestine from case 1, showing, in the lower portion, a marked reticulum cell hyperplasia with little maturation into monocytes, and a peripheral crowding of the remaining lymphocytes. Hematoxylin and eosin stain. × 200.

FIG. 33. In a kidney from case 6 there is a heavy interstitial infiltration with monocytes with partial obliteration of the renal parenchyma. Hematoxylin and eosin stain. × 50.

FIG. 34. Section of a vessel in the brain from case 2, showing an adventitial proliferation of reticulum cells and their maturation into monocytes. Hematoxylin and eosin stain. × 400.

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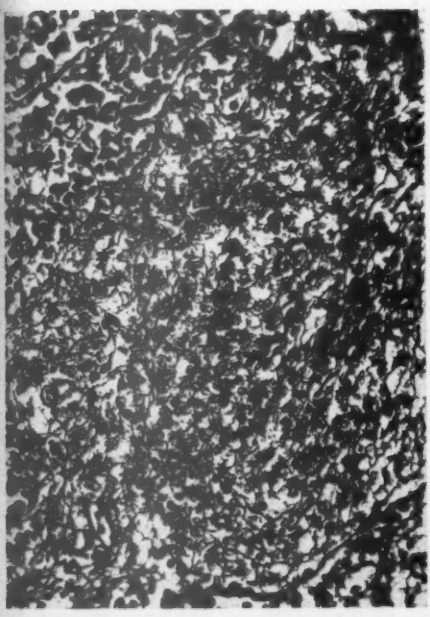

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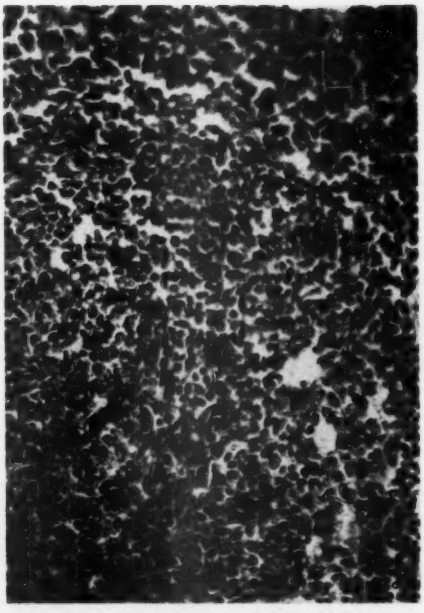

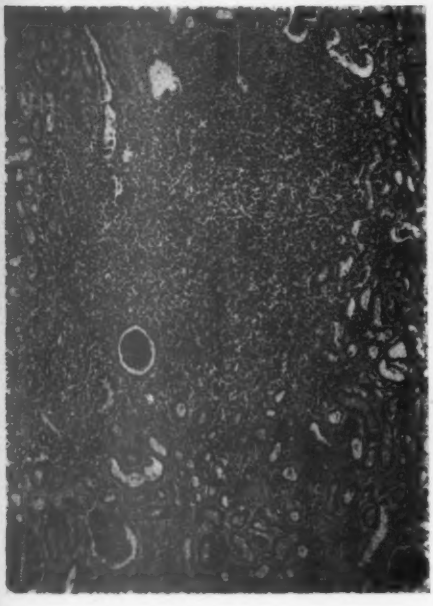

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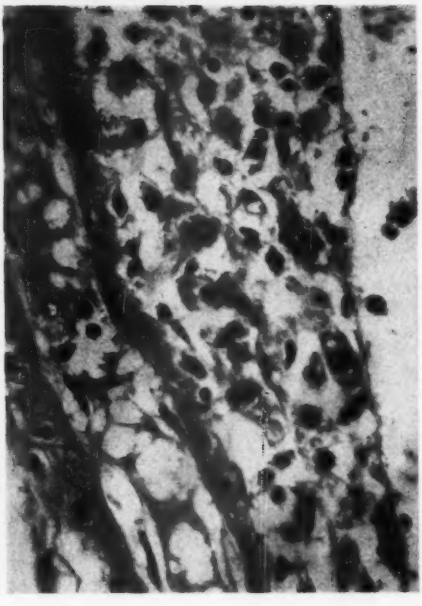

DRAINAGE OF THE PULMONARY VEINS INTO THE DUCTUS VENOSUS ARANTII

Report of a Case *

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Anomalous drainage of the pulmonary veins has been reported frequently in the literature since the first case of this type was described by Winslow [1] in 1739. In analyzing the literature up to 1942, Brody [2] classified the examples of anomalous pulmonary drainage into two groups: cases in which only a part of the blood from the lungs entered the right atrium by direct or indirect routes; and cases in which all of the pulmonary blood reached the right atrium. In the latter group of 23 cases, the course of the blood from lungs to right atrium was, in order of frequency, by way of anomalous vessels to the superior vena cava or its tributaries, to the coronary sinus, directly to the right atrium, or to the portal vein or the inferior vena cava.

Drainage of all of the blood from the lungs into the right atrium, either directly or indirectly, is believed to be incompatible with life. Brody [2] stated that apparently a minimum of 50 per cent of the aerated blood must reach the left atrium to sustain life. Compere and Forsyth [3] estimated that when as much as 75 per cent of the pulmonary blood empties into the left atrium there will be no recognizable cardiorespiratory symptoms.

Report of Case

The following is a case of congenital malformation of the heart and aorta associated with drainage of the pulmonary veins into the ductus venosus.

B. F., a white male infant, was born on January 23, 1945, following a normal pregnancy. The birth weight was 10 lbs., 1½ oz. At the time of birth the child was cyanotic and had the umbilical cord wrapped twice about his neck. In other respects the delivery was uncomplicated. After stimulation the child began to breathe and the color improved. Respirations were regular and the infant's color continued to improve. At the age of 17 hours no abnormalities were noted. At 30 hours the infant suddenly became dyspneic and cyanotic with slight retractions of the right side of the chest. The heart was thought to be enlarged bilaterally. Râles were audible in both lung fields. No heart murmurs were heard. The rectal temperature was 103° F. Subcutaneous fluids were given and the child was placed in an oxygen tent. In spite of oxygen cyanosis persisted. On the following day an immense cardiac dilatation to the left was noted, but no murmurs were heard. A diagnosis of a congenital defect of the heart was made. The infant was then taking and retaining formula feedings up to 1½ oz. and was restful and quiet

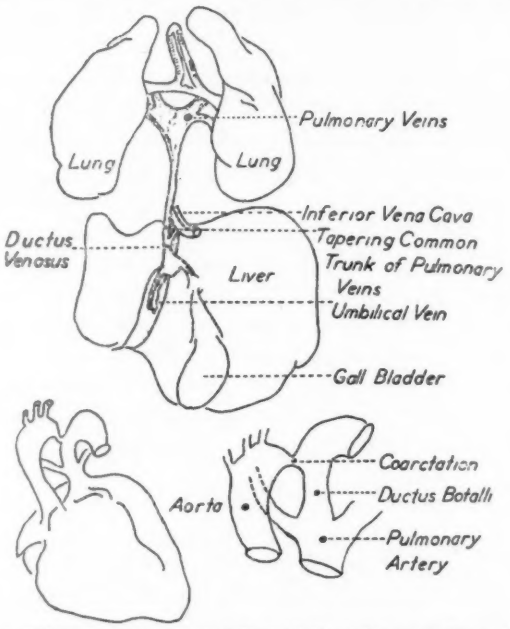
* Received for publication, January 1, 1946.

with oxygen. On the fourth day a prominent precordial systolic murmur was heard. This murmur persisted until the seventh day of life, after which it could no longer be heard. On the twelfth day the infant suddenly became very dyspneic, more deeply cyanotic, and expired after 45 minutes of severe respiratory distress.

Post-mortem examination revealed a cyanotic infant in whom all organs were normal except for anomalies of the heart, aorta and pulmonary veins. The superior and inferior venae cavae emptied into a right atrium which was normal except for a widely patent foramen ovale with a diameter of 13 mm. The right ventricle was greatly increased in size and its musculature was 5 mm. in thickness. Tricuspid and pulmonary valves were enlarged to a circumference of 43 and 33 mm., respectively, but had normal cusps. At the point of bifurcation of the pulmonary artery (diameter, 8 mm.) a patent ductus botalli, 7 mm. in length and 5 mm. in diameter, extended to the arch of the aorta. The left side of the heart was very small. No blood vessels entered the left atrium. The left ventricular musculature had a thickness of 3.5 mm. Mitral and aortic valves were normal in shape and each had a circumference of 22 mm. In the interventricular septum at a distance of 5 mm. from the apex of the heart was a small defect, 2 mm. in diameter (Fig. 1). The aorta, slightly increased in size (diameter, 9 mm.), had a normal configuration up to a point just distal to the left subclavian artery and just proximal to the point of entrance of the patent ductus botalli where its lumen was less than half its proximal size (diameter, 4 mm.) (Fig. 1 and Text-Fig. 1). The thoracic aorta distal to this coarctation was normal. The right pulmonary veins united near the hilus of the lung to form a common trunk. The veins from the left lung joined to form a common vessel in a similar manner. These two common trunks fused in the midline posterior to the heart (Fig. 2 and Text-Fig. 1) to form a single vessel, with a diameter of 6 mm., which descended from this junction anteriorly to the esophagus and aorta, and passed through the diaphragm in close proximity to the inferior vena cava to empty into the ductus venosus at a point 2 mm. above the obliterating umbilical vein. During its course downward this vessel decreased gradually in size so that at the level of the ductus venosus it had a diameter of only 2 mm. The ductus venosus was still patent with a diameter of 4 mm. It communicated freely with the inferior vena cava through a short trunk 8 mm. in length and 3 mm. in diameter (Fig. 2 and Text-Fig. 1).

Blood, oxygenated in the lungs, passed through the common trunk formed by the pulmonary veins into the ductus venosus arantii and thence into the inferior vena cava, with a probable backflow into the distal portion of the obliterating umbilical vein and the adjacent por-

tal vein. Oxygenated blood was thus mixed with the purely venous blood of the inferior vena cava. As it entered the right atrium the blood was again mixed with purely venous blood of the superior vena cava. From the right atrium the mixed blood passed into the pulmonary circuit, through the patent ductus botalli into the peripheral circulation, through the open foramen ovale into the small left atrium, and through the small interventricular defect into the left ventricle. The mixed blood from the left atrium and left ventricle passed into the aortic arch and



Text-Fig. 1. Diagrammatic representation of the autopsy specimen. Of note is the configuration of the aorta and pulmonary artery in the lower right corner.

its vessels, encountering resistance at the point of coarctation. No organ or part of any organ received purely arterial blood.

Discussion

A search through the literature revealed only one case of a somewhat similar anomalous pulmonary drainage (Table I). Ghon,[4] in 1916, described a 15-day-old infant in whom the pulmonary veins of each side united to form a common stem. These, in turn, formed another venous stem, 1 cm. in circumference, which lay anterior to the esophagus and aorta, proceeded inferiorly to pierce the diaphragm with the inferior vena cava and opened into a vessel which joined the still

patent ductus venosus arantii. Blood, arterialized in the lungs, passed by this route into the first part of the ductus venosus arantii and thence into the sinus of the left branch of the portal vein. The foramen ovale and ductus botalli were still patent.

Several cases have been reported in which the pulmonary drainage completely or incompletely emptied into blood vessels inferior in position to the right atrium. In such cases the embryological explanation of the anomalous drainage is probably similar to any which might be advanced for the case here reported.

TABLE I

Reported Cases

Author	Sex	Age	Drainage
Arnold[5]	F	15 weeks	Portal vein
Bochdalek[6]	M	4 days	Right upper, into superior vena cava; remainder of pulmonary veins into portal vein
Chassinat[7]	F	12 days	Right, into inferior vena cava
Cooper[8]		10 months	Right, into inferior vena cava
Geipel[9] (Right, half of thoracopagus)	F	5–6 month of pregnancy	Portal vein
Ghon[4]	M	15 days	Portal vein
Hu[10]	M	7 months	Portal vein (complete drainage of both lungs)
Munck[11]	M	3 months	Portal vein
Park[12*]	M	2½ months	Right, into inferior vena cava
Ramsbotham[13]		Newborn	Left, into subclavian vein; right, into portal vein
Terplan and Sanes[14]			Portal vein
Uchida[15]		Fetus	Inferior vena cava

* Case also reported by Brown.[16]

Zuckerkandl [17] (1881), by injections through the pulmonary vein, found that the injection mass filled not only the pulmonary veins themselves but also the bronchial veins and through them the venae azygos and hemi-azygos, and the mediastinal network of veins and, in many instances, reached even the postcaval and gastric veins. The pulmonary veins were seen to anastomose regularly with the veins of the mediastinal network. He concluded that anomalies might be caused by overgrowth or underdevelopment of capillary plexuses at a place where the vein in question should develop. He reasoned that pulmonary and systemic veins might merely be remnants of an original indifferent plexus from which drainage is developed to best suit the organ or tissue.

Evans,[18] in discussing theories of vascular systems, stated that vessels may develop secondarily in their own region from capillary plexuses. Blood vessels, according to this theory, develop in response to the need and the function to be performed by the organ in which the vascular system is found. The assumption that tissues and organs have a

rôle of importance in respect to plexuses and vessels may, he believed, be of assistance in explaining vascular patterns.

The present knowledge of the origin of the pulmonary veins is incorporated in two somewhat opposing views. One holds that the veins develop as a proliferation from the dorsal endothelial wall of the sinus venosus toward the lung buds, and is supported by investigators such as Federow,[19] Flint,[20] and Buell.[21] The other view is that the veins develop from communications between the sinus venosus and an indifferent plexus originally present in the region, and is supported by Brown [16] and von Möllendorff.[22]

In the view held by Brown,[16] no explanation is given of the source of the plexus, but many agree that the plexus about the lung bud is not specific, having been developed from the splanchnic plexus of the foregut. The conception that the pulmonary system is only a specially developed part of an indifferent plexus serves to explain communications between pulmonic and systemic circulations which are normally found and also those which constitute severe anomalies of the pulmonary veins. For example, an anastomosis (MacCready [23]) between the splanchnic plexus and the omphalomesenteric veins is a probable cause of a communication between the pulmonary and portal systems. Persistence of connection between pulmonary veins and the esophageal plexus and its connection with the developing inferior vena cava would account for a condition in which pulmonary veins open into the inferior vena cava.

Summary

In the case reported, pulmonary venous drainage was into the ductus venosus arantii. Only one somewhat similar case was found in the literature although several cases have been reported with complete or incomplete pulmonary venous drainage into vessels inferior to the right atrium.

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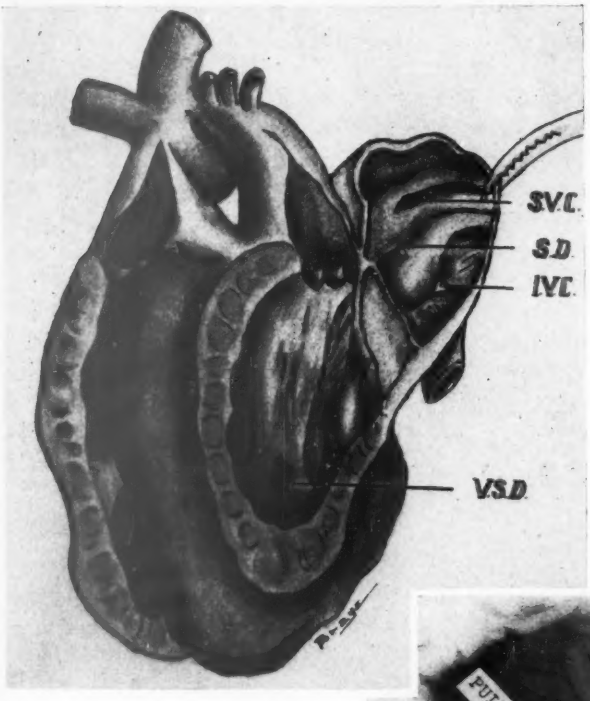
DESCRIPTION OF PLATE

PLATE 23

FIG. 1. Drawing of the heart as seen from the left, with the flap of the left ventricle removed and the left atrium reflected. S.V.C. = opening of superior vena cava; S.D. = location of atrial septal defect; I.V.C. = opening of inferior vena cava; V.S.D. = ventricular septal defect. Because of the almost complete absence of atrial septum, the view is distorted; the venae cavae actually empty into the right atrium.

FIG. 2. Autopsy specimen, posterior view, with the flap of the left ventricle reflected. String (small arrow on left) passes through the ventricular septal defect.


S.V.C.
S.D.
I.V.C.
V.S.D.

1

PULMONARY VEINS
LEFT VENTRICLE
INFERIOR VENA CAVA

2

GROSS CEREBRAL HEMORRHAGE AND VASCULAR LESIONS IN ACUTE TUBERCULOUS MENINGITIS AND MENINGO-ENCEPHALITIS *

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Much has been written during the past few years about the pathogenesis of tuberculous meningitis and many of the older concepts have been subjected to critical review. The literature dealing with its morphologic pathology, on the other hand, has been extremely scanty during the past 15 years. In fact, the first article in the American literature stressing the vascular changes was that of Winkelman and Moore [1] in 1940.

The older textbooks [2-4] are in general agreement that hemorrhages ("red malacia") are a common finding in tuberculous meningitis. Jakob,[5] for example, stated that not uncommonly there may be large extravasations of blood, hemorrhagic malacia, or a purpuric appearance. Statements to this effect are still to be found in recent textbooks,[6] although the experience of a number of present-day pathologists [7,8] does not confirm this impression. Stevenson [9] has stated that hemorrhages in tuberculous meningitis have become as rare as the conglomerate tubercle or the gumma.

The available literature shows a wide variation in the reported incidence of hemorrhagic phenomena. Hayem,[10] in 1868, described a case of hemorrhagic tuberculous encephalitis. Hoche,[11] in 1888, associated venous obliteration with isolated hemorrhages in one case. Gavazzeni [12] reported 2 cases in 1901. In 1902 Bombicci [13] reported 5 cases in infants or young children in the course of a lengthy study of hemorrhagic encephalitis. He noted that hemorrhages were either peripheral, usually frontal, or in the basal ganglia, and ranged up to 3 cm. in diameter. The hemorrhagic phenomena were ascribed chiefly to venous thrombosis due either to endothelial changes or "toxic factors." Aneurysmal dilatations were observed in one of the cases. Single cases were published by Vanzetti [14] in 1904 and by Grafe and Gross [15] in 1920. In the classic paper of Askanazy [16] in which the hyaline-fibrinoid degeneration now known by his name was first described, hemorrhages were found in 3 of 23 cases and were considered a rare phenomenon. Kirschbaum [17] mentioned hemorrhages in 5 cases. Winkelman and Moore [1] described no hemorrhages in the 5 cases which they presented in detail and did not mention their presence in the total of some 200 cases studied. It is also noteworthy that the Askanazy degeneration

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was not seen in their series. MacGregor, Kirkpatrick, and Craig [18] (1935) made no mention of hemorrhages. Biber [19] is the sole observer who found hemorrhages to be frequent: he demonstrated them in 15 of 17 cases.

Within the past year there have been autopsied at City Hospital, Welfare Island, 2 cases of acute tuberculous meningo-encephalitis which had gross hemorrhages and extensive vascular lesions of an unusual nature. They form the basis of this communication.

Report of Cases

Case 1

The patient, S.R. (no. 132792), was a Negress, 36 years old, who was admitted to the First Medical Division, service of Dr. J. Homer Cudmore, on April 1, 1945. Her chief complaint was severe headache of 8 days' duration. She believed that she had had some fever for the past month. There had been photophobia and a slight cough.

Examination revealed semistupor, moderate tenderness of the left lower quadrant of the abdomen, general hyperreflexia, the right knee jerk more active than the left, a positive Kernig sign, and a cross extensor reflex. The spinal fluid pressure was 370 mm. of water; cells, 450 per cmm. of which 60 per cent were small lymphocytes; sugar, 27 mg. per cent; protein, 62 mg. per cent; chlorides, 737 mg. per cent; Wassermann reaction, negative; gold-sol curve, 123443210; Levinson and tryptophane tests, positive. Culture yielded no growth. The white cell count was 9500; polynuclear cells, 68 per cent; lymphocytes, 26 per cent; eosinophils, 4 per cent; monocytes, 2 per cent.

The course was characterized by continuous low-grade fever, bradycardia, and progressive neurologic signs. The mental status showed a transient improvement but nuchal rigidity remained and nystagmus to the left was noted. Gradually the patient became apathetic, then irrational. Three days before death there were oculomotor paralysis and dilatation of the right pupil. Death occurred in coma on the 15th hospital day.

Examination of the spinal fluid obtained during the course of the illness showed a fall of the sugar to 18 mg. per cent and the chlorides to 684 mg. per cent. The Levinson and tryptophane tests remained positive. Cultures were sterile; a pellicle formed in one specimen.

Autopsy (no. 7221) was performed after the body had been embalmed. Only the findings for the brain are given in detail.

The entire base of the brain was covered by a thick, dense, yellow, fibrinous exudate which obscured the cranial nerves. There were numerous firm, stringy adhesions between the dura and leptomeninges of the frontal lobes. Over the surface were many minute, tubercle-like nodules, especially marked along the sulci. On sectioning, a small area of petechial hemorrhage was found just superior to the right inferior frontal gyrus and another on the antero-inferior aspect of the cerebellum. The area bordering the fourth ventricle was diffusely reddened by confluent petechiae (Fig. 1). There were a few more in the left putamen. The walls of the lateral ventricles had a finely granular

appearance, more extensive on the floor and extending upwards. The right ventricle was more affected than the left.

Microscopically, the meninges showed an extensive inflammatory reaction which varied from place to place. In the vicinity of some blood vessels there was intense serous exudation with only scanty cellular infiltration. In other areas large patches of fibrin were present. Some small areas showed an almost pure polynuclear infiltration, although a pleomorphic cellular response was far more common, usually consisting of polynuclear cells, small lymphocytes, plasma cells, phagocytes, swollen epithelioid cells, and débris. Frequently, small foci of incipient necrosis were found. Cellular débris was found in the center of nodules of epithelioid cells; in these areas tubercle bacilli were demonstrable. Only rarely were giant cells or characteristic tubercles to be found.

The meningeal vessels were extensively involved in this process. Intense perivascular infiltration was found everywhere. The adventitia was infiltrated by lymphocytes, plasma cells, and phagocytes. Occasionally the muscularis of the arteries showed disorganization of the architecture with coagulation necrosis or rhexis of the muscle fibers and infiltration with wandering cells or young fibroblast-like cells. In such areas the elastic fibers were either fibrillated, completely destroyed, or stained very poorly. Frequently the elastica interna was frayed, poorly stained, or completely torn, and the cellular infiltrate seemed to pour through this break and spread fanwise beneath the endothelium (Fig. 2). Incipient aneurysms were seen elsewhere (Figs. 3 and 4). Acid-fast bacilli were found in the adventitia or media of the affected areas, but none were found in the subintimal infiltrate. One subintimal tubercle with a giant cell was found, and within a single endothelial cell of an intact arteriole a juxtanuclear tubercle bacillus was seen. The subintimal infiltrate varied in character. In the least affected areas there might be only a single layer of cells, chiefly lymphocytes and occasionally polynuclear cells. In more severely involved regions macrophages and fibroblast-like cells also were found. No red cells or histiocytes were seen. Of great interest was the hyaline-fibrinoid degeneration of media and intima which was found in the smaller arteries and even in the smallest arterioles. The larger vessels (1.5 mm. in diameter and larger) showed pronounced changes of the elastica interna, but hyaline material was found in the intimal zone with great rarity. Almost all of the veins showed destruction of the elastic fibers; this was not constant in the arteries. The arterioles seemed to be more affected by the hyaline degeneration than the larger vessels. In many areas nearly every arteriole showed a dense, smudgy material

in the subintimal zone (Fig. 5); frequently there was thrombosis within the lumina. Occasionally a perivascular cellular infiltrate extended down and around these vessels, and this might be found even in the vicinity of capillaries (Fig. 6). An occasional artery was thickly mantled by large, uniform macrophages (Fig. 7). In the cerebellar tissue, particularly, destruction of the vascular walls was prominent; numerous thrombi were seen.

The brain tissue bordering on the meninges showed a narrow zone of infiltration in most areas. Gitter cells were observed and at times even the deeper tissues showed infiltrative and proliferative changes in areas where the inflammatory reaction extended deep into the sulci along the vessels. Acute edematous changes of a focal character also were present and there were large patches of proteinic exudate in the Purkinje layer of the cerebellum. In a few areas, characteristic tubercles of the cortex were found. The hemorrhages observed grossly were confined to those areas where vascular involvement and thrombosis were present. Sections from the frontal lobe showed hemorrhage around the vessels in Virchow-Robin's space and infiltrating the parenchyma in cuff-like fashion. In the cerebellum, vascular destruction was more marked, thrombi were common, and large areas of parenchyma were completely destroyed by hemorrhage.

The other pertinent anatomic findings were bilateral hydronephrosis, chronic cholecystitis, chronic salpingitis, and a Ghon tubercle of the left lower lobe of the lung. The diagnosis as to the brain was acute tuberculous meningitis and encephalitis.

Case 2

The patient, E.H. (no. 134514), a female cretin dwarf, 48 years old, was admitted to the First Medical Division, service of Dr. John Carroll, on July 25, 1945. The history was that of increasing stupor for 2 days, unaccompanied by pain, headache, or nausea. The only pertinent fact elicited in the past history was an attack of "flu" 4 months previously from which she "never fully recovered"; it had not been followed by residual cough or other respiratory symptomatology.

Physical examination revealed slight nuchal rigidity, positive Brudzinski sign, positive right Babinski reflex, and a negative Kernig sign. The spinal fluid was under a pressure of 310 mm. of water, very cloudy, and contained 800 cells per cmm., 95 per cent polynuclear cells, and 5 per cent lymphocytes. The protein was 251 mg. per cent; sugar, less than 5 mg. per cent; chlorides, 910 mg. per cent; Wassermann reaction, negative; gold-sol curve, 233455555; culture, sterile. The hemal leukocyte count was 10,000 per cmm., with 80 per cent polynuclear cells.

The temperature rose rapidly to 105° F. Eighteen hours after admission there were generalized tonic and clonic convulsions which could be controlled only by heavy sedation. Death occurred 20 hours after admission.

Autopsy (no. 7295) was performed 2 hours after death.

Examination of the brain showed a small amount of fine, fibrinous exudate over the base; otherwise there was little of note in the

meninges. The frontal lobes were extremely soft and had a bluish discoloration. On section, these lobes were almost completely destroyed and were converted into a soft hemorrhagic paste. At the periphery, the brain tissue was studded with innumerable hemorrhages ranging from pin-point to 3 mm. in diameter. The remainder of the cerebrum, midbrain, pons, medulla, and cerebellum showed nothing of note.

Microscopically, throughout the frontal lobes the intracerebral vessels showed extensive hyaline-fibrinoid degeneration (Fig. 8) involving nearly every vessel in the area. A large proportion of the vessels were thrombosed. Extensive hemorrhages surrounded most of these vessels and hematogenous pigment was present in the adjacent tissue. There was patchy, occasionally very dense, polynuclear infiltration around the affected vessels, and these cells also infiltrated the better preserved cerebral tissue nearby. There was also a concomitant infiltration with lymphocytes and gitter cells. In other parts of the brain no vascular changes were seen; there was no hemorrhage or infiltration with polynuclear cells, but lymphocytes and gitter cells were evident.

The leptomeninges showed only some slight edema in all regions examined. Only in those regions where the meningeal vessels were involved did more extensive changes occur. In those localized areas nearly all meningeal veins showed a hyaline-fibrinoid degeneration and, frequently, obliteration of the lumen by hyaline thrombi. The walls of the vessels sometimes were indistinguishable in such sections. Around the vessels and extending for a short distance into the leptomeninges there was a fairly dense exudate composed of fibrin, numerous multilobed polynuclear cells, occasional macrophages, and innumerable extravasated red cells. A few millimeters away from such an area the meninges were again apparently normal. The small arteries showed little change save for the infrequent areas of subintimal infiltration, chiefly with polynuclear and occasionally mononuclear cells. No giant cells or tubercles were seen anywhere, and only rarely did small clumps of epithelioid cells occur. The gray matter beneath these involved areas showed perivascular infiltration with round cells and numerous gitter cells, occasionally forming a layer just beneath the surface of the cortex. Even the white matter far from any meningeal lesion showed dense clusters of round cells about capillaries and small vessels. Acid-fast bacilli were found with extreme rarity.

Discussion

Hemorrhages

Several theories have been proposed to explain the occurrence of hemorrhages in uberculous meningo-encephalitis. Askanazy[16] believed that hemorrhages were due to venous thrombosis, as is com-

monly seen in hemorrhagic infarcts elsewhere. However, he thought that arterial occlusion might also be a causative factor. Biber,[19] on the other hand, believed that hemorrhage was due to diapedesis or to actual vessel rupture. The latter did not emphasize the rôle of thrombosis, possibly because none of the cases which he examined showed the fibrinoid degeneration which we now know may give rise to extensive thrombotic phenomena.

In our material it was quite evident that thrombosis with subsequent hemorrhagic infarction played the chief rôle. It was therefore impossible to decide whether ruptures of the small vessels were due to intrinsic damage to the vessel wall or whether they were part of the usual process of infarction with the wall giving way under the increased circulatory pressure. Although incipient aneurysms were seen in the walls of some of the larger arteries (Figs. 3 and 4), no actual rupture of such an area was found. Thrombosis was an outstanding feature of both cases. Thrombi were by far more common in the veins, and the hyaline (platelet) thrombi described by Biber [19] were seen frequently. In all areas where hemorrhages were present both arteries and veins showed multiple thrombi.

Vascular Changes

The acuteness of the lesions in both cases afforded an unusual opportunity to trace the evolution of the pathologic process. The attack on the vessels, always more destructive to veins than to arteries, clearly begins with an inflammatory reaction in the immediately periadventitial region. This observation confirms the experimental findings of Rich and McCordock [20] who, on injecting tubercle bacilli into the subarachnoid space, noticed that bacteria had a tendency to cluster around the vascular channels and attack them from without. This observation also modifies considerably the usual concept that the vessels are only incidentally involved in the meningeal inflammatory process.

In the very early stages of inflammation, as in case 2, the cellular response is chiefly polynuclear in character. Older lesions, as in case 1, approach more closely the typical cellular reaction of tuberculosis; *i.e.*, lymphocytes, plasma cells, macrophages, and occasional epithelioid cells. Rarely, a vessel may be mantled by large macrophages of a very uniform appearance. The character of the infiltrate varies with the rate of invasion of the vessel wall. In a rapidly destructive process the changes we have just described are found; in the slowly invading lesions tubercles, and eventually caseation, may appear.

The media is clearly the most resistant portion of the vessel wall. Even with a completely destroyed adventitia and thickly infiltrated intima the media is apt to be surprisingly well preserved. Occasional

cells may be seen apparently migrating through the media. When changes do occur, first a hyalinization and a slight basophilia of the muscle fibers appear. Later, there may be rhexis or even complete necrosis.

The elastica interna reacts to the inflammatory process by becoming fibrillar and fraying. Occasionally there is complete rupture. Some authors even have observed the torn ends to curl inward toward the vessel lumen,[19] a detail not seen in our material. Nor were we able to see any complete arterial ruptures or arterio-arterial fistulae as described by Biber,[19] although beginning aneurysmal formation was found.

The origin of the intimal lesions is probably manifold. Most commonly, the early intimal lesion consists of a pleomorphic cellular infiltrate beginning as a single layer of cells similar to the type of infiltration seen around the vessel. We were able to observe several lesions in which the perivascular infiltrate extended down through the media and the frayed elastica interna, and then seemed to spread out fanwise as soon as it reached the intimal zone. The tapering off of the density of the infiltrate as well as the absence of proliferative changes, considering the size of the lesion, is strongly suggestive of this sequence of events. Involvement of the intimal zone by way of the blood stream, which would correspond to the "tuberculous endarteritis" of Kirschbaum,[17] is a possibility not to be ignored. The existence of isolated intimal tubercles, as first shown in serial sections by Hektoen,[21] is strong evidence to support this concept. Moreover, in our case 1 we observed a tubercle bacillus actually within the swollen endothelial cell of an otherwise intact arteriole. This finding seemingly lends support to the hematogenous origin of some of the intimal lesions.

The hyaline-fibrinoid degeneration of Askanazy[16] was present to a marked degree in both of our cases. The peculiar fibrin-like material could be found both in the subintimal zone and in the media (Figs. 5 and 8). The frayed elastica interna was often the only remaining structure differentiating these two zones. Figure 8 showed the extent to which this change progressed in the parenchymal vessels of case 2. It is interesting to note that in case 2 this lesion was confined to the frontal lobes, as were all the hemorrhagic phenomena. The nature of this hyaline-fibrinoid degeneration remains obscure. Spang[22] noted that the ground substance in the histiocytes described by him gave the same positive Weigert fibrin reaction as the hyaline material of Askanazy. However, as there are no subintimal infiltrations of any kind in the areas where the fibrinoid degeneration is most prominent, at least in our material, this similarity in staining properties would

seem to be merely coincidental. Isibasi [23] agreed with Abrikosoff, whom he quoted, in believing this change to be allergic in nature and somewhat similar to periarteritis nodosa. However, Isibasi admitted that he has seen fibrinoid degeneration in nontuberculous meningitis. The resemblance of periarteritis nodosa to the fibrinoid degeneration seen in our material is rather faint. Whatever the cause of this lesion, it is interesting to note that it may be sharply localized in character, and that within such an area nearly every vessel may be affected. The intracerebral vessels were far more frequently affected than the meningeal vessels, and vessels of arteriolar or precapillary caliber were the site of predilection. Fibrinoid degeneration was observed in areas where the inflammatory exudate was so acute as to consist only of polynuclear leukocytes.

Summary and Conclusions

As illustrated by the two cases of hemorrhagic tuberculous meningoencephalitis forming the basis of this study, the vascular fibrinoid degeneration of Askanazy [16] is present in the subintimal zone and media of the involved vessels. The invasion of the blood vessels begins in the periadventitial region where the cellular process is, at first, chiefly polynuclear. The media is the most resistant portion of the vessel wall. The internal elastic lamina may become fibrillar and frayed, and may separate two zones of fibrinoid material. Fibrinoid degeneration is seen more frequently in intracerebral than in meningeal vessels.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

Figures 1 to 7 are from case 1; Figure 8 is from case 2.

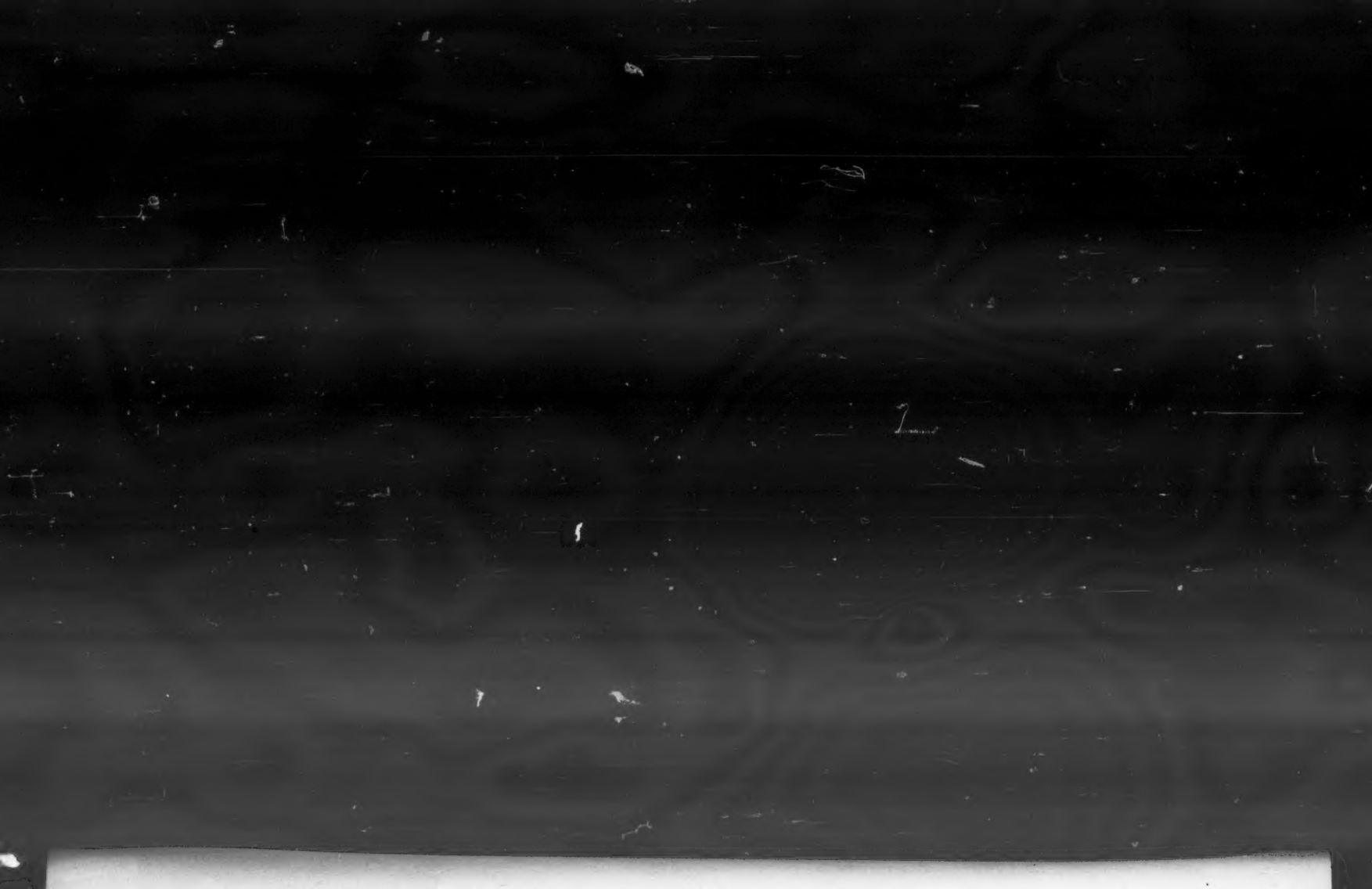
PLATE 24

FIG. 1. Right hemisphere. Of note are the clouding of the meninges by the thick exudate most marked at the base, the generalized congestion, and the confluent hemorrhages in the upper portion of the cerebellum.

FIG. 2. Large meningeal artery showing hyalinization and destruction of the vessel wall at one point (arrow), and fanwise spreading of the subintimal infiltrate from this area outward. Hematoxylin and eosin stain. × 75.

FIG. 3. Low-power view of a large artery showing the degenerative changes of the elastica interna with early aneurysmal formation in one area (arrow). Elastica and van Gieson's stains. × 85.

FIG. 4. High-power view of the same field as shown in Figure 3, showing destruction of the vessel wall, the subintimal granulation tissue, and the aneurysm. Hematoxylin and eosin stain. × 185.



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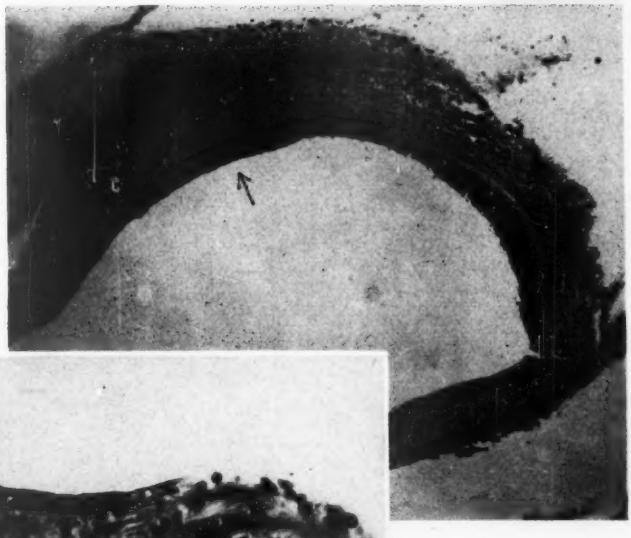

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PLATE 25

FIG. 5. Askanazy's degeneration in an arteriole. The fibrillar degeneration of the elastica is clearly visible in the lower portion of the vessel; most of the vessel wall is destroyed. Elastica and van Gieson's stains. × 380.

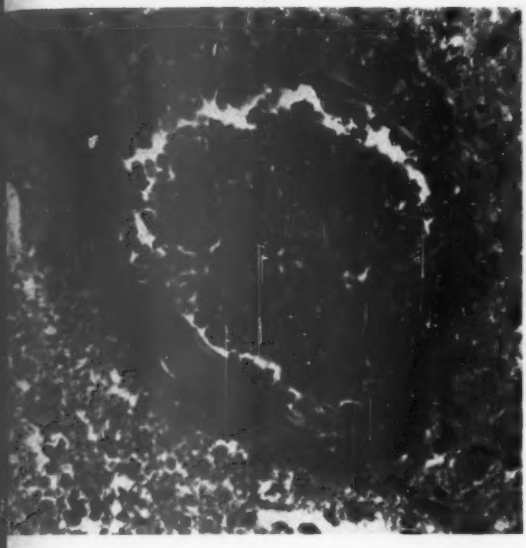
FIG. 6. Dense pericapillary infiltration and some swelling of the vessel wall, deep in the cortical tissue. Hematoxylin and eosin stain. × 380.

FIG. 7. Tangential section of an artery showing the thick mantle of large macrophages which surrounds the wall. Hematoxylin and eosin stain. × 380.

FIG. 8. Askanazy's degeneration in a vessel of precapillary size. There is also edema of the surrounding cerebral tissue. Elastica and van Gieson's stains. × 380.

AMER

Gold



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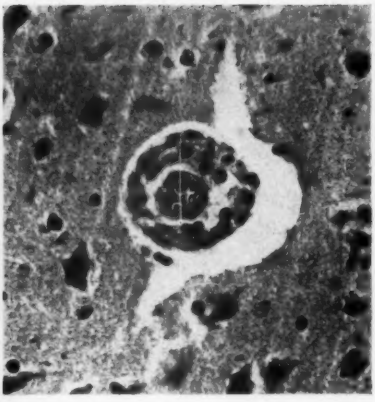

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STUDIES ON PERIARTERITIS NODOSA

II. The Rôle of Various Factors in the Etiology of Periarteritis Nodosa in Experimental Animals *

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In a previous report [1] we described the frequent occurrence of periarteritis nodosa in rats and dogs in which hypertension had been induced by the silk-perinephritis method.[2] These findings were in agreement with the results of several other investigators who likewise had observed that in hypertensive animals, periarterial lesions were often associated with sharply rising blood pressure curves.[3-5] It was pointed out by us [1] that periarteritis nodosa had not been found in any of the 38 control rats of the same age and that no report was found in the literature of the occurrence of typical lesions in any animals in which blood pressures and kidneys had been proved to be normal. The criteria used by us in diagnosing lesions of periarteritis nodosa were detailed in our previous report. In most of the animals the lesions were widely distributed, occurring in all organs except the lungs.

Recently Rich,[6,7] in reporting 7 cases of periarteritis nodosa in man, restated the theory first suggested by Gruber [8] that this condition may occur in human beings as the result of a generalized hypersensitive reaction to some foreign agent such as serum or sulfonamides. Rich and Gregory [9] found necrotizing vascular lesions in rabbits which had been sensitized to sterile horse serum, but were unable to produce similar lesions with sulfonamides.

Since the occurrence of periarteritis nodosa in our animals was strictly limited to those which had been subjected to the silk-perinephritis operation, and since lesions were found in some rats within 1 week after operation, experiments were made to determine what factor, or factors, are associated with the operation which are capable of initiating lesions of periarteritis nodosa.

In our previous experiments [1] silk-perinephritis was produced without recourse to strict aseptic technic. Most of the reports concerning the use of this procedure in rats have not described the use of sterile precautions.[2,10,11] The region where the silk is applied quite often (but not always) became infected. With the assistance of Dr. Irvin Gibby of the Bacteriology Department, bacteriologic studies of the pus which

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formed around the silk were made. Sometimes one organism was found, but often several organisms were present, most commonly staphylococci, streptococci, and *Salmonella enteritidis*. Cultures of these three types of organisms isolated from rats having typical lesions of periarteritis nodosa were used in some of the experiments to be described.

Since periarteritis nodosa was never found in any of the 38 control rats, it can be reasoned that the lesions found in these hypertensive rats developed as the result of one, or a combination, of the following factors: (a) hypersensitivity to a foreign body (silk); (b) hypersensitivity to one or more of the infectious agents often present around the silk; (c) arterial hypertension following renal manipulation; (d) release of some toxic substance from the kidney as a result of renal damage; (e) other unknown factors associated with the operation. Although several of the above possibilities cannot be conclusively ruled out by any experimental methods known at present, the results of the following experiments suggest that some of these factors have played no rôle in the production of periarteritis nodosa in these hypertensive rats.

In addition, an attempt was made to confirm the observations of Rich and Gregory [9] on rabbits made hypersensitive to horse serum. The plan, methods, results, and conclusions of the experiment on rabbits will be presented after detailing the experiments conducted on rats.

RATS

Methods

The rats used were albinos of both sexes, of the Wistar and Sprague-Dawley strains. The animals were about 6 months of age when operated upon and were fed only Purina Dog Chow Checkers and water *ad libitum*. All animals were weighed twice weekly and careful clinical notes were kept. In order to avoid the effect of frequent heating or anesthesia, required by indirect methods for measuring the blood pressure of a rat,[12,13] the only blood pressures measured in the present series were terminal ones, obtained by direct cannulation of the abdominal aorta [14] when the animal was sacrificed. Since the pressure at that time was found to vary with a number of conditions difficult to control, only limited significance has been attached to pressures thus measured.

The experiments on rats can be divided into three general types: (a) Experiments 1 and 2 consisted of introducing silk or infectious agents, or both, into the peritoneal cavity without manipulation of the kidney and without producing perinephritis; (b) In experiments 3, 4, and 5, silk alone, or silk plus some infectious material, was placed

around the kidney, producing perinephritis; (c) In experiment 6, silk plus infectious material was placed on the spleen, resulting in perisplenitis.

Experiment 1. Using sterile technic, 1 to 2 cm. bags of autoclaved silk or cellophane were placed in the abdominal cavities of 10 rats, care being taken to avoid the kidneys. These rats were sacrificed 12 weeks after operation.

Experiment 2a. A sublethal dose of a mixed suspension of three organisms, *Staphylococcus aureus, Streptococcus anhemolyticus* (gamma), and *Salmonella enteritidis* (all isolated from perinephric

TABLE I

Incidence of Periarteritis Nodosa in Rats Following Introduction of Silk or Infectious Agents, or Both, Distant to the Kidney

Experiment number	Experimental procedure	Periarteritis nodosa		
		Present	Absent	Questionable
1	Sterile silk or cellophane bags placed in abdominal cavity	0	10	0
2a	Intraperitoneal injection of mixed suspension of staphylococci, streptococci, and salmonellae	0	12	0
2b	Mixed suspension of the above 3 organisms in silk or cellophane bags placed in abdominal cavity	0	9	0
2c	Intraperitoneal injection of blood samples from rats showing typical lesions of periarteritis nodosa at autopsy	0	4	0
Total		0	35	0

abscesses of rats exhibiting typical periarteritis nodosa) was injected intraperitoneally into 12 normal young rats. These animals were sacrificed 32 weeks after injection.

Experiment 2b. Silk or cellophane bags containing small amounts of a suspension of the same three organisms were placed in the abdominal cavities of 9 rats, care being taken to avoid the kidneys. These rats lived from 6 days to 12 weeks following operation.

Experiment 2c. Large blood samples (4 to 5 cc.) from each of 4 rats exhibiting gross nodular lesions at autopsy were injected intraperitoneally into 4 normal young rats. These rats were sacrificed from 4 to 12 weeks following injection.

As indicated in Table I, periarteritis nodosa was not found in any of the 35 rats included in experiments 1 and 2.

Experiment 3. Using sterile technic, autoclaved silk was wrapped around one kidney of each of 26 rats; the other kidney was removed. After periods varying from 11 days to 48 weeks, these animals died or were sacrificed. Eleven rats had developed gross or microscopic evi-

dence of infection in the region where the silk had been placed. The incidence of lesions of periarteritis nodosa in the infected and non-infected rats is shown in Table II.

Experiment 4. One kidney of each of 75 rats was wrapped with silk and on the silk was placed a drop of a pure culture, diluted with broth or liquid agar, of one of the same three organisms used in experiment 2. The other kidney was either removed or in some cases left un-

TABLE II

Effect of Various Infectious Agents on the Incidence of Periarteritis Nodosa

Experiment number	Material used to produce perinephritis	Silk on one kidney and silk on other kidney, or other kidney removed			Silk on one kidney, other kidney left untouched			Total
		Periarteritis nodosa			Periarteritis nodosa			
		Present	Absent	* Questionable	Present	Absent	* Questionable	
3	Sterile silk	4	9	1	0	1	0	15
	Sterile silk which became infected	3	7	0	0	1	0	11
4	Silk infected with streptococci	0	4	1	0	8	1	14
	Silk infected with staphylococci	7	3	2	0	7	0	19
	Silk infected with salmonellae	7	3	2	0	0	0	12
	Silk infected with mixed organisms	2	2	0	0	0	0	4
5	Silk rubbed on the floor	12	4	2	0	0	0	18
Data from authors' previous report[1]	Clean silk applied without any sterile precautions	27	34	9	0	2	1	73
Total		62	66	17	0	19	2	166

* Some arteries not normal, but no full-blown necrotizing exudative lesions. Some of these cases probably are of very early or very late lesions.

touched. One-third of these rats died of peritonitis within a few days following these procedures, but 49 survived from 10 days to 45 weeks. Suspension of the organisms in liquid agar was found to give lower initial mortality. In 15 animals a mixture of all three organisms was placed on the silk; of these only 4 rats survived the acute stage and were studied.

Experiment 5. Silk contaminated by rubbing it on the laboratory floor was placed around one kidney of each of 18 rats, the other kidney being removed. This method was found to result in a high incidence of periarteritis nodosa and the initial mortality was low. These animals lived from 3 days to 40 weeks.

The results of experiments 3, 4, and 5 are shown in Table II. For

purposes of comparison, the data from our first study [1] are also included in Table II.

Experiment 6. Silk contaminated by rubbing it on the laboratory floor was wrapped around the spleen in 18 rats, producing a perisplenitis comparable to the perinephritis which resulted when similarly contaminated silk was placed around the kidney. These animals lived from 7 to 30 weeks. At autopsy the spleens were imbedded in thick collagenous hulls which contained infected pus pockets similar to those found in perinephritis. None of these 18 rats presented lesions of periarteritis nodosa either grossly or microscopically.

TABLE III

Incidence of Periarteritis Nodosa in Rats Following Various Operative Procedures

Type of operative procedure	Periarteritis nodosa			Total
	Present	Absent	Questionable	
Silk or infectious material, or both, distant to the kidney	0	35	0	35
Silk-perinephritis	62	85	19	166
Silk-perisplenitis	0	18	0	18
Controls; no operation	0	43	0	43
Total	62	181	19	262

A group of 43 rats were not operated upon but served as controls in regard to sex, age, strain, species, diet, and environment. A summary of the incidence of periarteritis nodosa following the various types of operative procedures and in the controls is shown in Table III.

Results in Rats

In experiments 1 and 2 in which silk or infectious agents, or both, were introduced into the abdominal cavities of 35 rats not adjacent to the kidneys, no periarterial lesions were noted.

When sterile silk was wrapped around the kidney and it remained sterile, or when it was infected with streptococci, there were positive lesions found in only 4 of the 29 animals comprising these groups. Three of the 4 (all originally sterile silk) were animals which survived for long periods after operation. At autopsy there was no pus, but extensive adhesions were found, which may be taken as evidence that infection may have been present at some previous time.

Of the 21 animals which had silk placed around one kidney and the other kidney left untouched, not one showed positive lesions of periarteritis nodosa grossly or microscopically. This is quite significant since in 7 of these the silk had been infected with staphylococci, while 7 of the 12 rats in which silk infected with staphylococci was placed

around one kidney and the other kidney removed had periarteritis nodosa.

Infecting the silk around one kidney with staphylococci or salmonellae or by rubbing it on the floor, and then removing the other kidney, produced lesions of periarteritis nodosa in 60 per cent of the surviving rats. On the other hand, when silk, contaminated by rubbing it on the floor, was wrapped around the spleen, a marked perisplenitis developed but no gross or microscopic evidence of periarteritis nodosa was found in any of the 18 rats studied.

These results in rats can be summarized as follows: Periarteritis nodosa occurred in a majority of the animals in which the silk around the kidney was infected with staphylococci, salmonellae, or by rubbing on the floor. No such lesions were found in rats in which the spleen was wrapped in silk similarly infected, or in which one kidney was left intact even though the other kidney was similarly infected, or in rats in which silk or similar infectious agents, or both, were introduced into the peritoneal cavity distant to the kidneys.

RABBITS

Experiment 7. Eight young albino male rabbits were divided into two groups. Those of the first group, 5 animals, were injected with 10 ml. of normal horse serum (sterile) per kg. of body weight; the second group of 3 rabbits served as untreated controls. Blood pressures of all animals were determined in triplicate on both ears by means of a Grant-Rothschild capsule.[15] Determinations were made every second day, beginning a week before the injections were made and continuing until the animals were sacrificed. Once before and three times during the period following injection of horse serum the animals were bled from the heart by cardiac puncture. Albumin-globulin ratios were measured and approximate determinations of the globulin fractions were made by means of a fractional precipitation-turbidimetric procedure.[16] Five to 6 days after the initial injection of horse serum several of the treated rabbits had flushed ears, a phenomenon previously described by Fleisher and Jones.[17] On the 28th day all rabbits were skin-tested with the original horse serum and all treated rabbits gave positive skin tests varying in degree from moderate to marked. The untreated rabbits all gave negative skin tests. On the following day all animals were given an intravenous injection of 3 ml. of the same lot of sterile horse serum. All survived and were sacrificed 2 hours after injection. Autopsies were performed and tissues from all animals were prepared for microscopic examination.

Results in Rabbits

All 5 rabbits which were injected with horse serum developed hypersensitivity, indicated by the positive skin tests and the presence of abnormal amounts of pseudoglobulin in their plasmas. Nevertheless, none of the 8 rabbits had lesions remotely suggestive of periarteritis nodosa and no evidence of glomerulonephritis was found in the kidneys of either the treated or control groups. The blood pressures, which were determined repeatedly, never varied more than 10 mm. of Hg from the original pre-treatment levels. Thus in this small group of rabbits we were unable to elicit lesions of periarteritis nodosa by producing hypersensitivity to sterile horse serum.

Discussion

The occurrence of periarteritis nodosa in animals has been attributed chiefly either to the production of a sharply rising hypertension [1,3-5] or to the development of hypersensitive reactions to known or unknown antigens.[9,18,19] The following observations lend support to the first explanation:

1. Several authors have reported the frequent occurrence of periarteritis nodosa in animals in which marked kidney lesions or hypertension, or both, have been induced. Wilson and Byrom [5] produced hypertension by placing a silver clip on one renal artery; Friedman, Jarman, and Klemperer,[3] by wrapping one or both kidneys with cotton; we,[1] by wrapping one or both kidneys with silk; Ham,[20] by feeding massive doses of vitamin D which led to renal calcification; and Selye and Pentz,[4] by injecting desoxycorticosterone into unilaterally nephrectomized rats. Despite the diversity of the methods utilized in producing an elevated blood pressure, all of these investigators found that from 40 to 75 per cent of their animals developed typical lesions of periarteritis nodosa. It would appear to be more than coincidence that 95 per cent of all cases of this condition which have been reported in young rats occurred following the experimental production of hypertension by one of the several methods listed above.

2. We found in our previous study [1] that animals presenting lesions of periarteritis nodosa at autopsy had a significantly higher mean blood pressure level than did a similar group of hypertensive rats in which these lesions were not found. This difference was apparent within 1 month after operation. Similar differences in the blood pressure curves between animals with periarteritis nodosa and those without were noted by Wilson and Byrom,[5] Friedman, Jarman, and Klemperer,[3] and Selye and Pentz.[4]

Reasoning from the above theory that hypertension plays a rôle in the pathogenesis of periarteritis nodosa, one might logically ask—why, then, is periarteritis nodosa not found in every animal in which hypertension is experimentally produced? The most plausible answer would seem to be that periarteritis nodosa is not the result of hypertension alone, but rather is a very frequent complication in experimental hypertension whenever the method used in inducing the hypertension results in a sharply rising blood pressure curve, with peak pressures averaging between 200 and 240 mm. of Hg or even higher. From the limited pathologic data available it would appear that methods of inducing hypertension in rats which result in mean blood pressure levels of less than 200 mm. of Hg do not as a rule give rise to a significant incidence of periarteritis nodosa.[21,22]

The following facts are in favor of the theory that periarteritis nodosa is the result of hypersensitivity to known or unknown antigens: (1) The experimental production of hypertension usually has necessitated the introduction of a foreign body or infection, or both; (2) Rich and Gregory [9] found necrotizing vascular lesions in rabbits made hypersensitive to sterile horse serum.

Against the theory that periarteritis nodosa occurs in animals solely as a result of hypersensitivity can be listed the following facts:

1. Although the incidence of periarteritis nodosa is high in hypertensive rats, nevertheless it has been stated by Rich and Gregory,[9] and is generally accepted, that: "The well-known high resistance of the rat to anaphylactic sensitization would render this animal a particularly unsuitable subject for the experiment. Most investigators have been unable to produce either anaphylactic shock or local anaphylactic tissue damage (Arthus phenomenon) in the rat."

2. If periarteritis nodosa is the result of hypersensitivity either to the silk, cellophane, or cotton, or to the infectious agents introduced either purposefully or otherwise along with the silk, then the introduction of these materials at some point other than around the kidney should give rise to these periarterial lesions. We consider it highly significant that when silk or infectious material, or both, were placed by us in the abdominal cavity not adjacent to the kidney, or were placed adjacent to the testicles by Friedman, Jarman, and Klemperer,[3] no lesions were found. When silk which had been rubbed upon the laboratory floor was placed around the spleens of 18 rats, producing a marked chronic suppurative perisplenitis, no lesions of periarteritis nodosa occurred. Yet when silk similarly rubbed on the floor was placed on one kidney of each of 18 rats and the opposite kidney removed, 12 animals developed typical lesions of periarteritis nodosa.

3. Although data concerning the distribution of lesions of periarteri-

tis nodosa in various organs of rats and human beings will be presented in detail in a subsequent report, the fact that typical periarteritis nodosa was not found in the pulmonary arterial tree appears highly significant. Its complete absence in the lungs is difficult to account for on the basis of differential hypersensitivity, and can much more easily be explained by the fact that the blood pressure in the pulmonary circuit is relatively low and is not significantly raised in experimental hypertension.[23]

4. In a small but carefully controlled experiment we were unable to produce typical periarteritis nodosa in rabbits by sensitizing them to sterile horse serum. In a much larger series of rabbits made hypersensitive to horse serum, Fox and Jones [24] also were unable to confirm the observations of Rich and Gregory.[9] Most of the animals presenting periarteritis nodosa, reported by Rich and Gregory, were also found to have glomerulonephritis. This lesion is commonly associated with hypertension, but no blood pressure determinations were made on their rabbits. Therefore, evaluation of the rôle of hypersensitivity in the pathogenesis of experimental periarteritis nodosa should be reserved until it has been demonstrated that periarteritis nodosa can be produced by hypersensitizing animals in which the blood pressure remains within normal limits and in which the kidneys present no complicating lesions.

Summary

Experiments have been performed to determine what factor, or factors, associated with the silk-perinephritis method of producing hypertension in rats, are capable of initiating lesions of periarteritis nodosa.

Typical lesions occurred in a majority of unilaterally nephrectomized animals in which the silk around the remaining kidney was infected with staphylococci or salmonellae, or by rubbing the silk on the floor. No such lesions were found in rats in which the spleen was wrapped in silk similarly infected, or in which one kidney was left intact even though the other kidney was similarly infected, or in rats in which silk or similar infectious agents, or both, were introduced into the peritoneal cavity distant from the kidney.

No lesions of periarteritis nodosa were found in 5 rabbits which had been made hypersensitive to sterile horse serum.

The available data support the hypothesis that periarteritis nodosa is associated with sharply rising hypertension, and tend to refute the theory that this condition is the result of hypersensitivity to known or unknown antigens.

We wish to thank Mrs. Elizabeth D. Goldsmith and Mrs. Norma R. Fetter for their technical assistance.

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THE NONPORTAL DISTRIBUTION OF THE TRABECULAE IN DIETARY CIRRHOSIS OF RATS AND IN CARBON TETRACHLORIDE CIRRHOSIS OF RATS AND GUINEA-PIGS *

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The relationship of choline, cystine, and methionine to necrosis and cirrhosis of the liver in rats has been investigated in a number of laboratories.[1-7] These two conditions now have been clearly identified and established as separate entities.[2,6] However, there still exist differences of opinion as to the architectural relationship of the connective tissue trabeculae in the cirrhotic livers produced by dietary deficiency of choline. Most workers have described the fibrous tissue as being formed in, or emanating from, the portal areas. This description corresponds to that of portal cirrhosis in man. In a previous report describing the histogenesis of dietary cirrhosis in rats,[8] we stated that the trabeculae of ceroid and connective tissue formed along the hepatic venules and only later abutted on portal areas. This interpretation does not permit the classification of the process as portal cirrhosis.

In order to test this interpretation, it was decided to continue the investigation of this subject using precise means of locating the position of the trabeculae. For this purpose, hepatic or portal veins were injected with a charcoal mass similar to that used by Mall [9] in his classic investigations on the hepatic lobule.

The centrolobular distribution of necrosis and fatty degeneration of the liver in CCl_4 intoxication is well recognized. However, the location of the subsequently formed connective tissue trabeculae is a controversial point. Therefore, the histogenesis of this process was investigated in the same manner as that of the dietary cirrhosis.

Experimental Procedure

Forty-three albino rats at weaning were started on diet no. 545 which had the following composition: Leached casein, 4 per cent; cystine, 0.5 per cent; cod-liver oil, 2 per cent; Wesson oil, 3 per cent; Osborne and Mendel salt mixture,† 4 per cent; corn starch, 86.5 per cent. A supplement of 100 μg. of thiamin chloride, 50 μg. of riboflavin, 20 μg. of pyridoxine, 50 μg. of calcium pantothenate, and 1 mg. of

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†Osborne, T. B., and Mendel, L. B. The use of soy bean as food. *J. Biol. Chem.*, 1917, 32, 369–387.

nicotinic acid was given to each rat daily. All rats received 20 per cent alcohol as a source of fluid. In order to obtain livers showing variable degrees of pathologic alteration, the rats were killed after having been under experimental conditions from 50 to 150 days. The livers were injected with charcoal gelatin mass through either the hepatic or portal veins.

Nineteen adult albino rats, weighing from 248 to 388 gm., were injected subcutaneously with carbon tetrachloride twice a week. The dose of carbon tetrachloride was 0.05 cc. per 100 gm. of initial body weight. The same dose was continued for the duration of the experiment regardless of the weight changes. These rats were fed an *ad libitum* diet of stock pellets and water. The rats were killed at intervals from 21 to 87 days and the livers were injected with charcoal gelatin mass.

In another experiment, guinea-pigs fed a stock vegetable diet received repeated subcutaneous injections of carbon tetrachloride at various dosage levels. Some of these animals developed cirrhosis. The livers of 9 were injected with the mass.

Preparation of Injection Mass. The injection mass is made just before use by dissolving 8 gm. of gelatin in 100 cc. of hot water and adding 20 gm. of animal charcoal. While the animal is being prepared for injection, the mass is kept in a conical glass in a warm water bath (about 55° C.). The charcoal is kept in suspension by use of a glass agitator rod of propeller type, powered by a small electric motor having an adjustable friction reduction gear.

Preparation of Animal and Injection of Liver. The animal is killed with chloroform and attached to a small wooden autopsy board. Anterior abdominal and thoracic walls are removed, and absorbent cotton is folded over the cut ends of the ribs to prevent injury to the liver. An obstructing tie is made around the inferior vena cava at any convenient point below the liver, usually just above or below the right renal vein. When it is desired to inject the hepatic veins, a glass cannula is inserted into the thoracic inferior vena cava through a slit made in the right auricle and tied in place. The cannula is attached by a 6 inch length of small rubber tubing to a 100 cc. syringe containing warm saline solution. After cutting the portal vein, gentle pressure on the syringe plunger is exerted to wash the blood from the liver. Most of the blood is quickly removed by this method. In some livers the blood remains in a few patches. The removal of this blood is effected by gently stroking the liver surface with the moist fingertips while continuing the irrigation. After the blood has been washed out, the charcoal gelatin mass is injected through the same tubing by replacing the syringe

containing saline solution with a 10 cc. syringe containing the mass. In the transfer of syringes, care should be taken to avoid the inclusion of air bubbles. The amount of pressure necessary to inject the hepatic tree is slight and is controlled by observation of the degree of filling of the subcapsular terminal branches of the hepatic veins. When the veins are filled, additional pressure may rupture the hepatic vein at the hilus. Injection of the intrahepatic portal veins is accomplished in a similar manner, the cannula being tied in the portal vein in any accessible location. The inferior vena cava is cut in the thorax to allow escape of the blood and saline solution. Since the terminal branches of the portal vein do not reach the surface of the liver, only a grayish color results from the injection. The injection is stopped when the system will not, with gentle pressure, receive more of the mass. Livers are injected either by hepatic or by portal veins, never by both. Although some of the gelatin passes through the hepatic lobules, the carbon does not, and is limited to the portal or hepatic systems, as the case may be. It should be noted here that occasionally some venous branches will not be filled by the mass.

After the injection is completed, the cannulized vessel is clamped and the entire liver removed and placed in the fixative, which in this study was either 10 per cent formalin or Zenker's fluid. The specimen is then placed in the refrigerator to accelerate the hardening of the mass. After 1 to 2 hours, the livers may be sliced at 3 to 4 mm. intervals, and fixation continued at room temperature.

For this study all tissue was embedded in paraffin. Sections were stained for connective tissue by Mallory's aniline blue and van Gieson's picrofuchsin methods. Lillie's modification of the di-ammine silver hydroxide method * was used for demonstrating reticulum.

Distribution of Trabeculae in Dietary Cirrhosis

In the dietary cirrhotic livers in which the charcoal gelatin mass was introduced through the hepatic vein, the injected veins were regularly included in, and connected by, the connective tissue trabeculae. In the livers injected through the portal veins, the injected veins were usually not in, or connected by, the trabeculae.

As previously reported,[8] the early deposition of fat was most marked about hepatic venules. Ceroid soon appeared in liver cells and in groups of phagocytes about hepatic veins and venules. These phagocytes became incorporated into loosely fibrous trabeculae. The trabeculae

* Lillie, R. D. A simplified method of preparation of di-ammine-silver hydroxide for reticulum impregnation; comments on the nature of so-called sensitization before impregnation. *Stain Technol.*, 1946, **21**, 69–72.

formed not only about the central vein but also along the sublobular and large branches of the hepatic veins. In fact, in some instances the first increase in connective tissue to be noted appeared around these larger vessels, more particularly near the hilar portion of the lobes.

In comparing portal versus hepatic distribution of fibrous tissue, one should compare similar levels of the two systems. This tends to lessen confusion in interpretation of the histologic picture. The point where the recognition of the terminal radicles of portal and hepatic veins is easiest is in the subcapsular layer of lobules. Here, where sections are made perpendicular to the surface, alternating central veins and portal veins of the same order can often be seen. It was apparent in the present study that the trabeculae coursed along these hepatic veins and reached and merged with the capsule, as is evident in Figure 2. It is significant that hepatic veins normally reach the capsule, whereas portal veins do not, a fact reported by Mall,[9] Figure 1. In deeper lobules the trabeculae are also seen to connect injected hepatic veins. Parts of three lobules with their constituent noninjected and uninvolved portal areas are shown in Figure 6. Part of the apparent disproportionate size of the portal and hepatic veins is due to distention of the latter by the injection mass.

Some trabeculae seen in livers injected through the hepatic vein did not contain the mass. This may be explained by the failure of the mass to reach the area due to narrowing of the vein or blockage at a lower level by an aggregate of charcoal particles. To determine how trabeculae were related to portal areas, it was necessary to study cirrhotic livers in which the charcoal mass was introduced through the portal vein. Figures 7 to 10 show injected portal veins in uninvolved portal areas. These lobules are surrounded by trabeculae containing no mass. Such relationship was found with regularity in those livers in which the cirrhotic process had not progressed to the point of extreme disarrangement of lobular architecture. Even in these, isolated nodules of hepatic parenchyma often showed uninvolved portal areas with injected veins (Fig. 11). In livers showing fairly advanced cirrhosis, portal areas were found at the margins of trabeculae and sometimes incorporated within them (Fig. 13). These portal areas contained vessels of relatively large size and obviously were not related directly to the functional liver unit. It was clear in some instances that a trabecula passed by, abutted on, or enclosed a portal area because destruction of one or more lobules had brought hepatic and portal vessels close together. Also the larger hepatic veins pursued an independent course to their exit from the liver,[10] a circumstance which in some instances brought the larger hepatic and portal vessels in close relationship at certain

points. Such a condition would account for the inclusion of an occasional portal area in a trabecula which was primarily related to the hepatic vein. In the extensively involved livers, some trabeculae extended to and incorporated the larger portal areas, and when such occurred the fibroblasts of these areas undoubtedly contributed to the new growth of connective tissue. In areas where almost complete replacement of parenchyma had taken place, usually near the hilus, injected portal veins of medium and large size were seen irregularly scattered throughout the ceroid and connective tissue mass.

Proliferation of small bile ducts is a feature of this cirrhosis which varies greatly in degree in different rats. It is usually prominent only in some of the animals showing moderately severe or severe damage. The presence of these ducts in the trabeculae served to confuse the picture and make interpretation difficult. That these bile ducts were present in the trabeculae, which in this study were shown to follow and connect hepatic veins, was evident; and the explanation for their presence was also evident in some preparations. These small ducts were seen extending from the portal area through the lobule between cell cords and reaching the trabeculae (Fig. 11). In most instances many more ducts were present in the trabeculae than were seen traversing the lobules. That these represented growth with tortuosity or budding in their abnormal location seems reasonable. In those livers where cirrhosis had proceeded to complete replacement of certain lobules, the reason for the presence of bile ducts in the proliferated connective tissue was obvious. This was seen particularly toward the base of the lobes and in diffuse or patchy subcapsular areas.

Distribution of Trabeculae in Carbon Tetrachloride Cirrhosis of the Liver

In hepatic cirrhosis induced by carbon tetrachloride the fatty degeneration and necrosis in both rats and guinea-pigs occurred around the hepatic veins, mainly the terminal branches (centrolobular) (Fig. 12). The trabeculae of fibrous tissue formed in the same location. These trabeculae and their relationship to the injected hepatic veins are shown in Figures 15 to 20. Their distribution was essentially the same as that already described in more detail for dietary cirrhosis.

The demonstration that the connective tissue trabeculae in cirrhosis due to CCl_4 follow and connect hepatic veins rather than the portal areas simplifies the understanding of the pathogenesis of this condition. The connective tissue proliferates in the same areas where damage to the liver takes place. Since this is true, it is unnecessary to assume, as has been suggested, the presence of an autolysate which travels in the

lymphatics from areas of necrosis to the portal areas, and there stimulates the production of connective tissue.

Discussion

The hepatic lobule is generally considered as having a terminal radicle of the hepatic vein at its center and being bounded peripherally by an imaginary line passing through the associated portal areas. This conception is based largely on the fact that in swine a connective tissue capsule is found in this location, thus enclosing a mass of liver cells—the hepatic lobule. In the seal, hepatic lobules are also well demarcated from one another, but in this animal it is a different lobule that is outlined.[11] Microscopically, it is seen that the hepatic veins are at the periphery, where, as lacunae or rectilinear venous sinuses, they form walls of separation of the portal lobule. Theile [12] demonstrated in the dog and rabbit the presence of cleavage planes between portal lobules. This was done by crushing, and washing the livers of these animals in a stream of water. When treated thus, the whole system of lobules became isolated and were clustered around the branches of the portal veins. The foregoing are cited to indicate that from a standpoint of architecture and comparative anatomy it should be possible to produce cirrhosis of the liver in which the trabeculae form at the periphery of either the hepatic or portal lobule, the type produced probably depending on the location of the injury.

The concept that the connective tissue proliferation occurs in the same location as the injury is supported by the fact that in carbon tetrachloride cirrhosis of rats and guinea-pigs, fatty degeneration and necrosis occur about the central veins and the fibrous tissue trabeculae form in and connect these areas. Further support for this concept is found in dietary cirrhosis. In the development of this condition, the deposition of fat usually occurs first, and is most pronounced, around the hepatic veins. This is followed by the accumulation of ceroid and the formation of connective tissue strands which follow and connect these hepatic veins.

Summary

Albino rats at weaning were placed on a cirrhosis-producing diet and killed after 50 to 150 days. The livers were injected through the portal or hepatic veins with charcoal gelatin mass to mark these structures effectively in the microscopic preparation. Histologic study showed that the fatty deposition, ceroid accumulation, and fibrous trabeculation primarily followed and connected hepatic veins. In livers showing marked alteration, the trabeculae sometimes coursed by, or abutted on,

large portal areas. However, even in these livers the portal areas comparable in level to the centrolobular veins were not primarily related to the trabeculae.

In other experiments, cirrhosis of the liver of rats and guinea-pigs was produced by the repeated subcutaneous administration of carbon tetrachloride. The livers were injected with the charcoal gelatin mass and studied histologically. The connective tissue trabeculae occurring in these livers were primarily related to hepatic veins and showed essentially the same distribution as that seen in the dietary cirrhosis.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

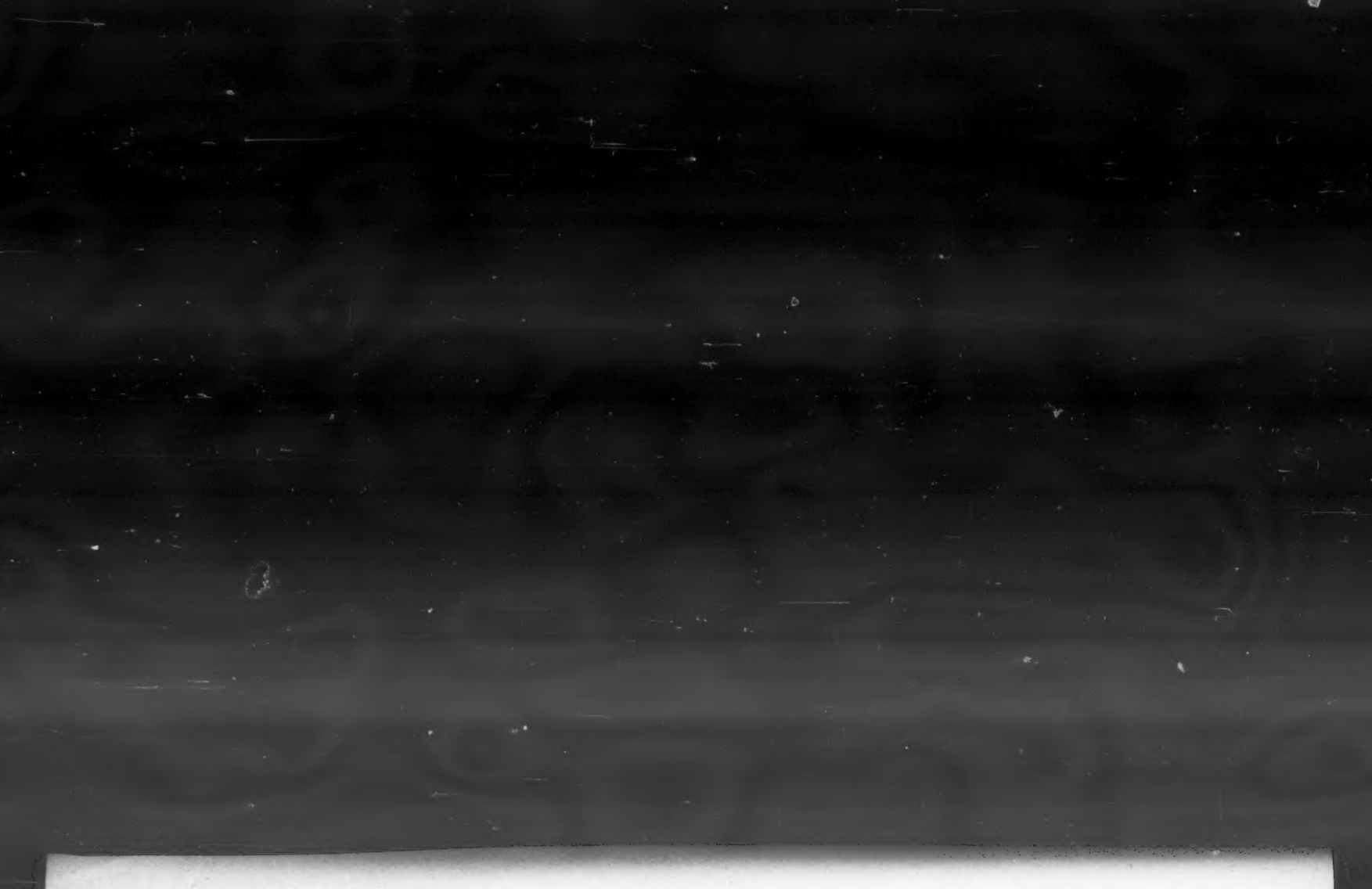
PLATE 26

FIG. 1. Dietary cirrhosis, rat. Gross photograph of liver injected with charcoal gelatin mass through hepatic vein. No gross evidence of cirrhosis.

FIG. 2. Dietary cirrhosis, rat. Liver showing increase in connective tissue around the injected hepatic vein. The connective tissue follows the vein to, and blends with, the capsule at the point of retraction. Reticulum stain. × 80.

FIGS. 3, 4, and 5. Dietary cirrhosis, rat. Relationship of trabeculae to the injected hepatic veins is shown. Ceroid appears in the trabeculae as small refractile globules. Van Gieson's stain. × 50.

FIG. 6. Dietary cirrhosis, rat. Injected hepatic veins incorporated in, and connected by, trabeculae. Parts of three lobules are shown; of note are the uninjected portal veins outlined by reticulum. Reticulum stain. × 50.



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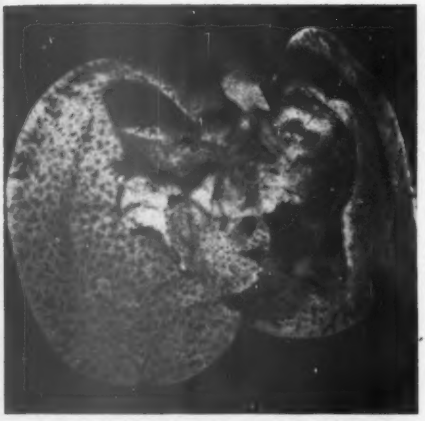

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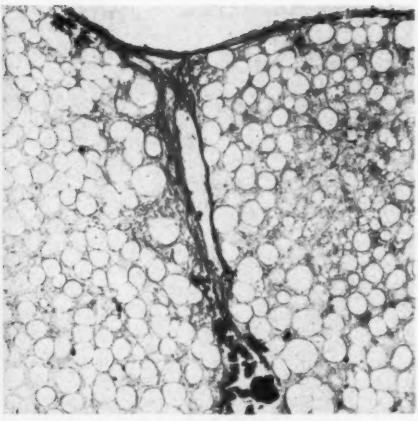

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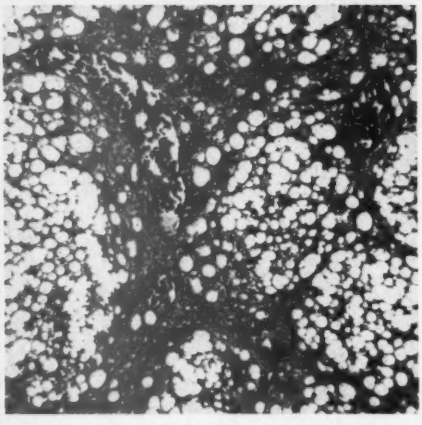

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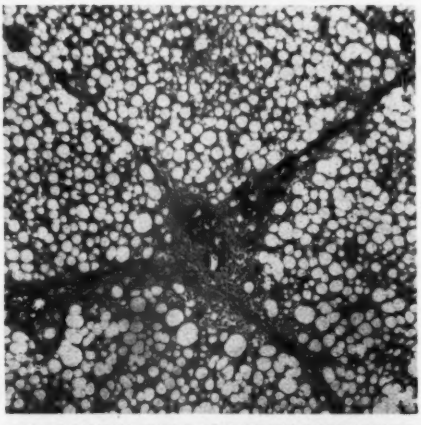

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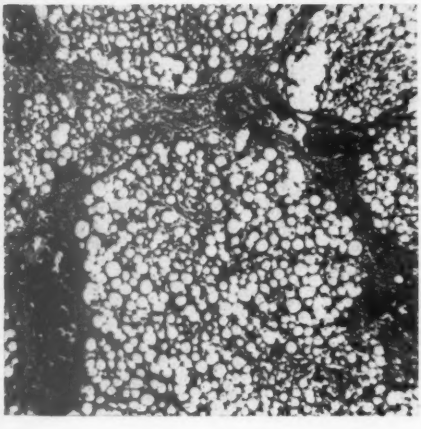

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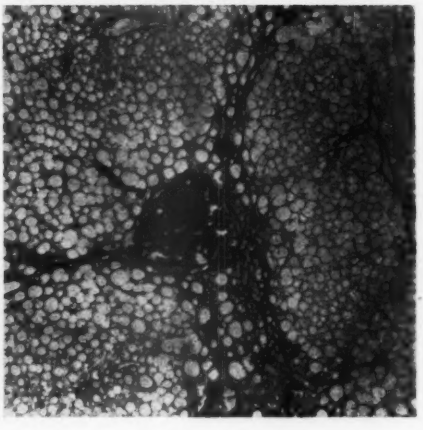

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Ashburn, Endicott, Daft, and Lillie

Nonportal Trabeculae in Experimental Cirrhosis

PLATE 27

FIGS. 7, 8, and 9. Dietary cirrhosis in a rat, with portal injection. Mallory's aniline blue stain. × 50.

FIG. 10. Dietary cirrhosis in a rat, with portal injection. Picrofuchsin stain. × 50.

FIG. 11. Dietary cirrhosis in a rat, with portal injection. Isolated nodule of liver. Hematoxylin and eosin stain. × 250.

FIG. 12. Carbon tetrachloride cirrhosis in a rat, with portal injection. Frozen section, stained with oil red O. × 50.

FIG. 13. Dietary cirrhosis in a rat, with portal injection. Narrow expansions from the thick trabeculae abut on the large portal area. The small portal area is unrelated to the proliferated connective tissue. Mallory's aniline blue stain. × 50.

FIG. 14. Carbon tetrachloride cirrhosis in a rat, with portal injection. Van Gieson's stain. × 50.

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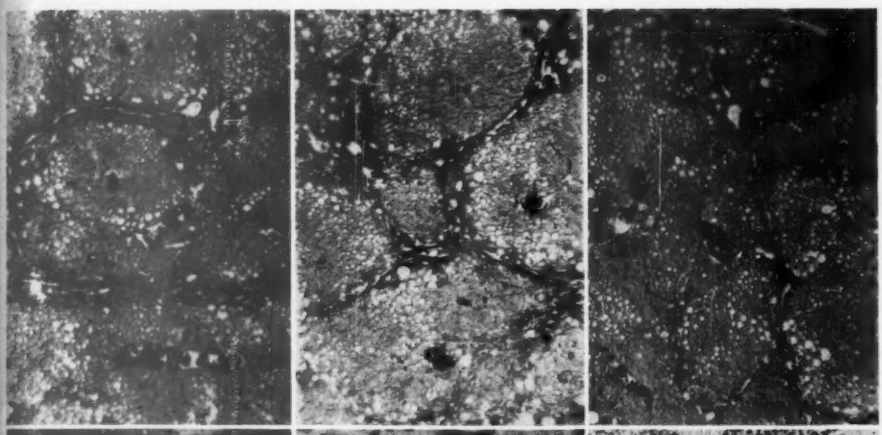

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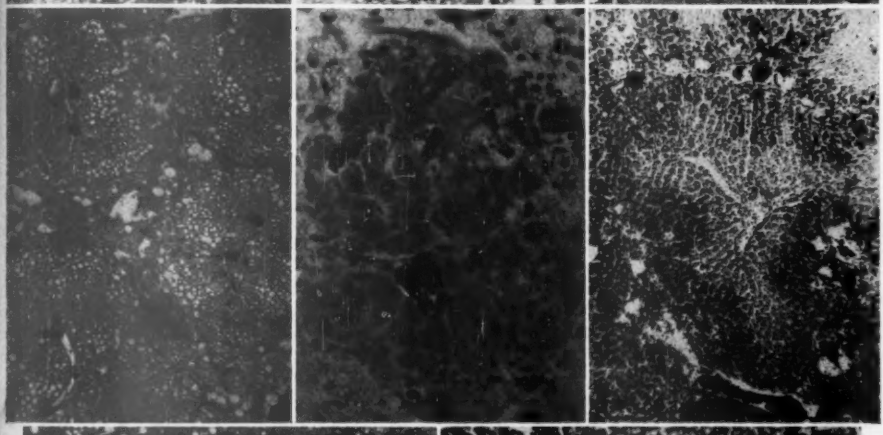

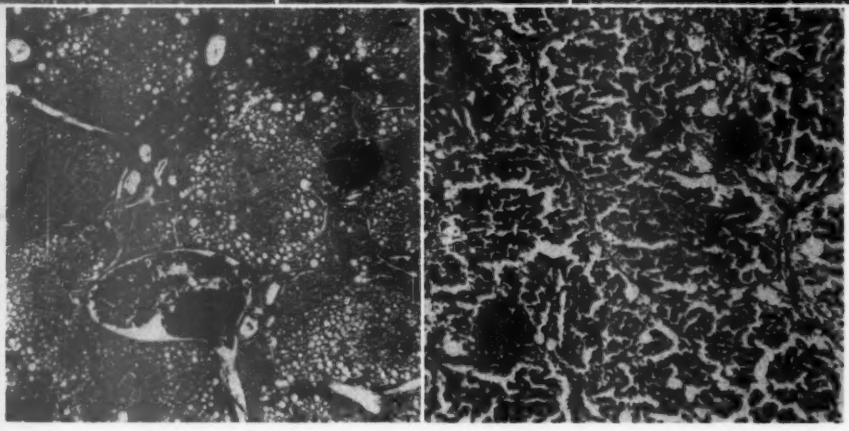

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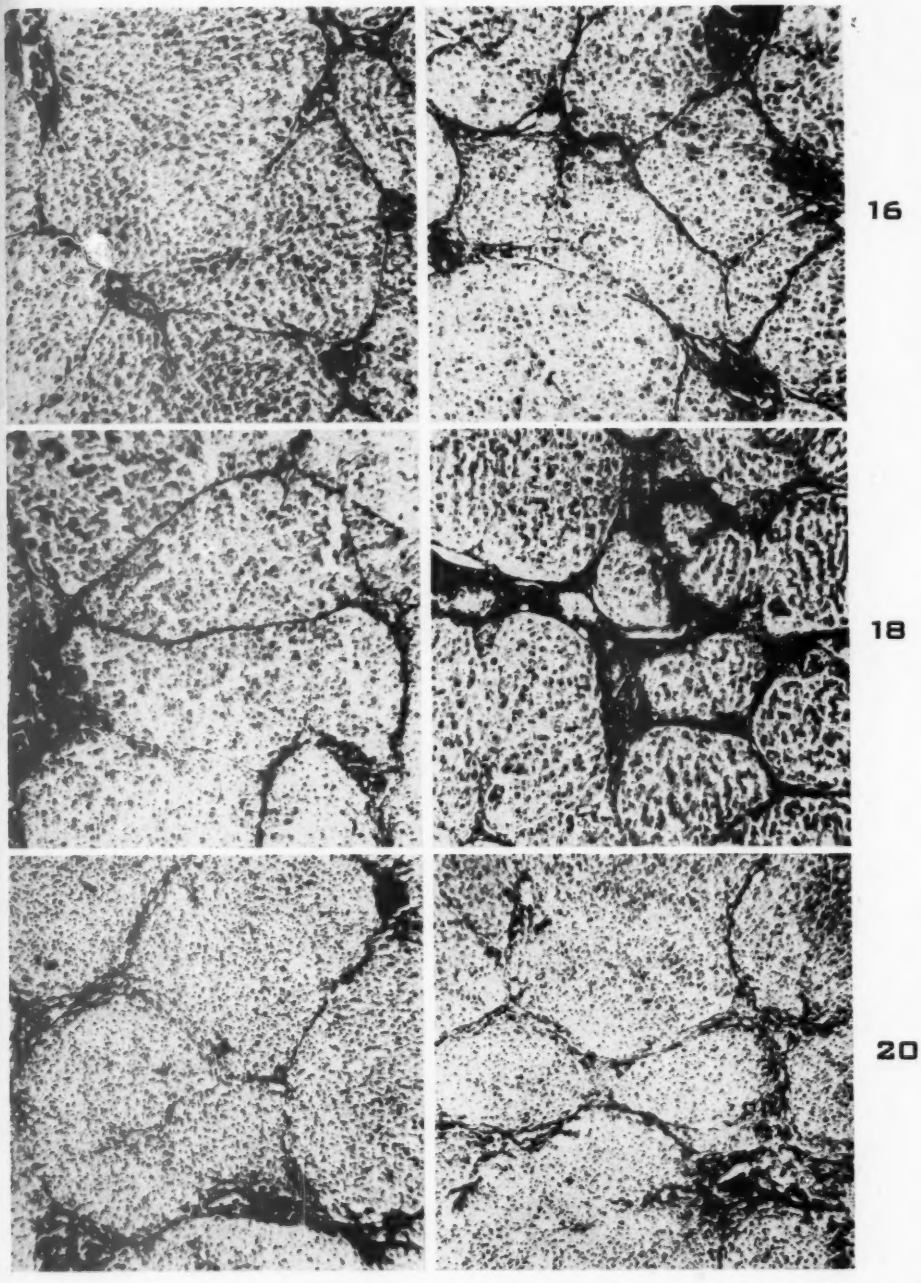
Ashburn, Endicott, Daft, and Lillie Nonportal Trabeculae in Experimental Cirrhosis

PLATE 28

FIGS. 15, 16, 17, and 18. Carbon tetrachloride cirrhosis in a rat; hepatic veins injected. There is considerable alteration of lobular architecture. Trabeculae follow and connect hepatic veins. Van Gieson's stain. × 50.

FIGS. 19 and 20. Carbon tetrachloride cirrhosis in a guinea-pig; hepatic veins injected. Injected veins are included in, and connected by, thin trabeculae. Van Gieson's stain. × 50.



Ashburn, Endicott, Daft, and Lillie Nonportal Trabeculae in Experimental Cirrhosis